Assessing the permissiveness of complex bacterial communities towards conjugal plasmids – Development of a novel method

Klümper, Uli; Riber, Leise; Sannazzaro, Analia; Dechesne, Arnaud; Musovic, Sanin; Hansen, Lars H.; Sørensen, Søren J.; Smets, Barth F.

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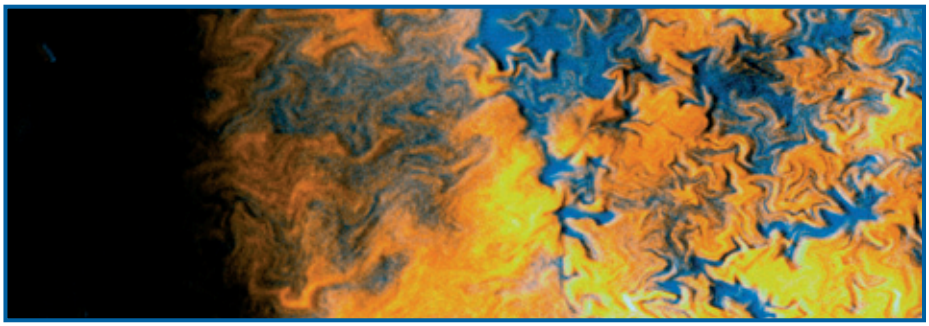
Bacterial Genetics and Ecology

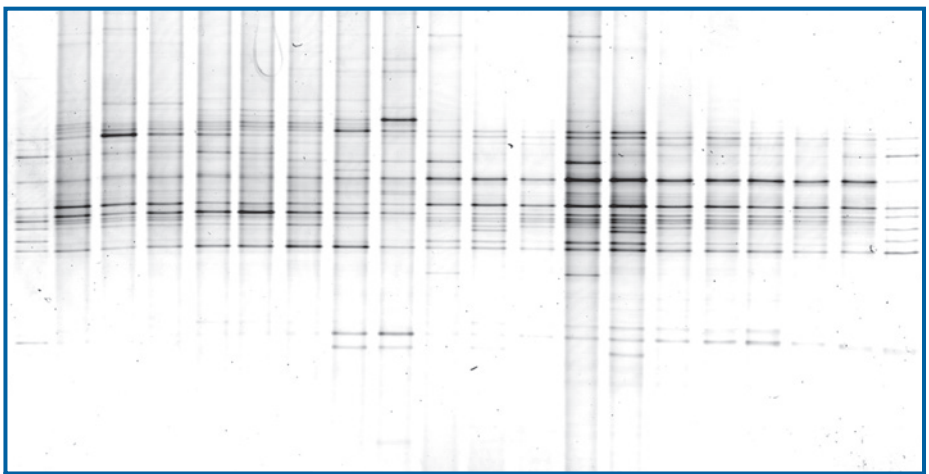


NETWORKING AND PLASTICITY OF MICROBIAL COMMUNITIES: THE SECRET TO SUCCESS

9–13 June 2013

Ljubljana, Slovenia

www.bageco2013.org



Scientific Programme Overview

	Sunday, 9 June 2013	Monday, 10 June 2013	Tuesday, 11 June 2013	Wednesday, 12 June 2013	Thursday, 13 June 2013
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		08:30–10:05 **Session I** Prokaryotic evolution and horizontal gene transfer I p. 11	08:30–10:05 **Session III** Microbial interactions with eukaryotic hosts I p. 13	08:30–10:05 **Session V** Beneficial microbes p. 15	
09:00					09:00–10:35 **Session VII** Microbial responses to anthropogenic impacts and approaches to alleviate them I p. 16
10:00		Industrial exhibition & coffee break	Industrial exhibition & coffee break	Industrial exhibition & coffee break	
		10:35–12:30 **Session I** Prokaryotic evolution and horizontal gene transfer II p. 11	10:35–12:10 **Session III** Microbial interactions with eukaryotic hosts II p. 13	10:25–12:00 **Session VI** Ecophysiology p. 15	Industrial exhibition & coffee break
11:00					11:05–12:40 **Session VII** Microbial responses to anthropogenic impacts and approaches to alleviate them II p. 16
12:00		Industrial exhibition & lunch break	Industrial exhibition & lunch break	Industrial exhibition & coffee break	
				12:30–13:45 Round Table p. 15	
13:00					Farewell
			13:10–14:45 **Session IV** Microbial community diversity and ecosystem function I p. 13		
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				14:15–18:00 Tour Postojna Caves p. 36	
15:00			Industrial exhibition & coffee break		
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Venue and Date
Grand Hotel Union Executive
Miklošičeva cesta 1
1000 Ljubljana (Slovenia)
9–13 June 2013

Conference Homepage
www.bageco2013.org

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Editor
Ines Mandic-Mulec
Editorial Deadline
30 May 2013

Welcome to BAGECO 12 in Ljubljana

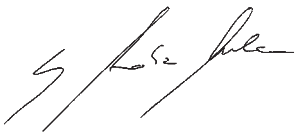
Dear Colleagues

Recent years have brought us striking discoveries in the field of bacterial genetics and ecology that sprung from rapid advances in sequencing technologies and "omics" approaches, bioinformatics, microscopy and various analytical techniques. These advances have allowed us to gather vast amounts of data, marvel at the remarkable diversity of microbial communities, culture previously unculturable organisms and discover novel ecosystem functions. These rapid developments now call for critical evaluation of the vast knowledge obtained and for hypothesis-driven research that will lead to novel concepts and connections and will be addressed during the **12th Meeting on Bacterial Genetics and Ecology (BAGECO 12)**.

We are pleased to welcome some of the most renowned scientists in this field to give presentations on recent advances in prokaryotic evolution and horizontal gene transfer, sociomicrobiology and microbial community networking, microbial interactions with eukaryotic hosts, drivers of microbial community diversity and ecological outcomes, beneficial microbes, and microbial responses to anthropogenic impacts and biotechnological advances that may alleviate them.

Your abstract contributions have helped us prepare a very exciting programme encompassing 36 oral and 230 poster presentations and we would like to thank you for supporting BAGECO and joining us in Slovenia. Our hope is that the meeting will provide the best possible environment to discuss your data with colleagues, to establish new networks and collaborations and to enjoy social networking with the scientific microbial ecology community in the wonderful city of Ljubljana.

We are more than delighted to welcome you to Ljubljana.



Ines Mandic-Mulec
Conference Chair

General Information

General Information

Venue
Grand Hotel Union Executive
Miklošičeva cesta 1 • 1000 Ljubljana (Slovenia)

Date
9–13 June 2013

Homepage
You will find up-to-date information on our website at **www.bageco2013.org**.

Registration Fees

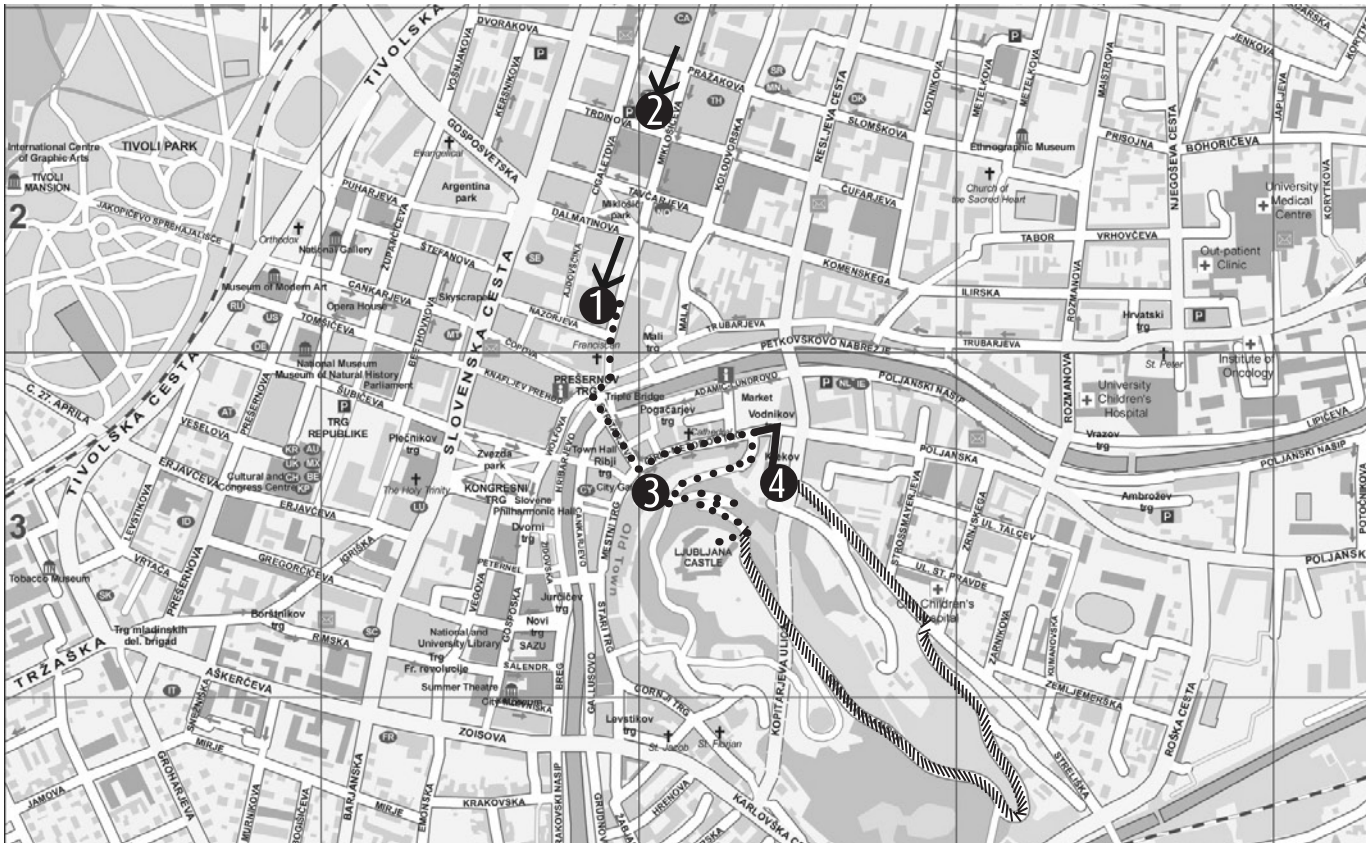
Regular	545 EUR
Student*	445 EUR

*Please provide proof of your student status.

Tour to Postojna Caves, 12 June 2013	20 EUR
Conference dinner, 12 June 2013	50 EUR

Parking
The Grand Hotel Union offers a secured on-site garage with 120 parking places, which can be found at the Grand Hotel Union Business. Parking costs 17 EUR per vehicle per night.

City Map



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••••••••• Grand Hotel Executive – Ljubljana Castle – shortest way by foot (don´t use when raining!)
Grand Hotel Executive – Ljubljana Castle – easier way by foot
Way to funicular station

❶ Grand Hotel Union
❷ Central Hotel
❸ Ljubljana town hall
❹ Lower funicular station

General Information

Name Tag
Attendants and registered accompanying persons will receive a name tag after registration. Admittance to the congress and industrial exhibition is only allowed for those with a name tag. Name tags should be worn at all times. Name tags for exhibitors will be given to the exhibit personnel.

Evaluation
Please turn in your completed and legible evaluation form to the Check-In on the last day. We are always striving to provide high congress quality. This goal can only be reached with your help, your active participation and constructive criticism.

Check-In
Check-In can be found at the entrance of the Grand Foyer. You will receive all ordered tickets for our social programme, conference dinner, your congress bag and tags for the poster contest.

Wardrobe
An unguarded wardrobe is located diagonally opposite our Check-In and free of charge. The congress organisation assumes no liability.

Media Check-In
Our Media Check-In can be found in our plenary hall.

Industrial Exhibition
There is an accompanying industrial exhibition. The exhibitors are looking forward to welcoming you and to presenting their comprehensive range of innovative products. For a detailed layout of the exhibition plan see page 35.

Opening Hours

	Sunday	Monday	Tuesday	Wednesday	Thursday
Industrial Exhibition		09^{00}–18^{00}	08^{30}–18^{00}	08^{30}–14^{00}	09^{00}–11^{00}
Poster Exhibition		15^{25}–18^{30}	17^{00}–20^{00}		
Check-In	14^{00}–18^{30}	08^{00}–18^{30}	08^{00}–19^{30}	08^{00}–14^{30}	08^{30}–13^{00}
Media Check-In	14^{00}–18^{30}	08^{00}–18^{30}	08^{00}–19^{30}	08^{00}–14^{30}	08^{30}–13^{00}

Internet
Computers with internet access are available next to the Check-In desk. Additionally wireless internet (WLAN) is available for all congress attendees:
Network bageco2013
Password bageco

Language
The congress language is English.

General Tips for Authors and Presenters

Submitting Your Presentation/Technical Information
Please prepare your presentation in 4:3 aspect ratio.

A presentation notebook with a PDF reader and MS Office PowerPoint 2010 will be provided. The use of personal notebooks is possible upon agreement. However, it may interrupt the flow of the programme in the lecture hall. Please provide an adapter for VGA if necessary.

A notebook, presenter and laser pointer are available at the speaker's podium in the lecture hall. If necessary a technical supervisor can help you.

Please note that certain encodings for video and audio files could lead to problems. Please visit our Media Check-In for further information in advance.

Media Check-In
The Media Check-In is located in our plenary hall.

Please submit your presentation at the Media Check-In desk in the plenary hall ideally the day before your presentation, but no later than 2 hours before the presentation should begin. You may view and/or edit your presentation.

For submission, please use a USB flash drive, CD or DVD disc, which should not be protected with software.

Speaking Time
Please prepare your presentation for the allotted amount of time. Chairs and moderators may interrupt should you overrun your time limit. Speaking time is assigned as follows (speaking + discussion time):

1. Keynote	30 + 5 minutes
2. Plenary lectures	15 + 5 minutes

Poster Sessions
Posters should be no larger than DIN A0 (84.1 cm x 118.9 cm). Poster pinboards are 120 cm x 150 cm. They are only to be used with the designated pins. Pinboards will be numbered. You will find your poster number in the programme book on pages 18–33. Posters should be erected on 10 June by 15^{00} hrs and removed on 12 June by 18^{00} hrs. All posters that have not been removed by then will be considered as waste. Poster presenters are asked to be present during their poster sessions.

Scientific Programme • Sunday, 9 June 2013

17^{00}–17^{30} **Opening of BAGECO 12**

Welcome address
Peter Raspor, President of Slovenian Society of Microbiology

Welcome address
Ines Mandic-Mulec, Conference Chair BAGECO 12

Musical intermezzo
Anže Petrač, Vocalist

17^{30} Keynote Lecture
01 • On becoming a symbiont – comparative genomic analyses of chemosynthetic symbionts of hydrothermal vent mussels and their closest free-living relatives
Nicole Dubilier (Germany)

from 19^{00} **Welcome reception at Ljubljana town hall** (see page 36)

Scientific Programme • Monday, June 10, 2013

08^{30}–10^{05} **Prokaryotic evolution and horizontal gene transfer I**
Chair Kornelia Smalla (Germany)

08^{30} Keynote Lecture
02 • Trends and barriers to lateral gene transfer during microbial evolution
Tal Dagan (Germany)

09^{05} 03 • Biofilms and mobile genetic elements – when the agenda of the mobile gene pool is to socialize
Jonas Stenløkke Madsen (Denmark)

09^{25} 04 • Incorporating signatures of gene evolutionary history into phyletic profiling for large-scale inference of microbial gene function
Fran Supek (Spain)

09^{45} 05 • Ecological and evolutionary views on how bacterial diversity buffers pathogen invasion in soils
Joana Falcão Salles (Netherlands)

10^{05}–10^{35} Industrial exhibition and coffee break

10^{35}– 12^{30} **Prokaryotic evolution and horizontal gene transfer II**
Chair Marco Bazzicalupo (Italy)

10^{35} Keynote Lecture
06 • Horizontal genetic transfer and the origin of bacterial species
Frederick M. Cohan (United States)

11^{10} 07 • Bacterial species that are defined by genes but not by ecology
Peter Young (Great Britain)

Scientific Programme • Monday, June 10, 2013

11^{30} 08 • How have terrestrial thaumarchaea really evolved?
Cécile Gubry-Rangin (Great Britain)

11^{50} 09 • Molecular authentication of the phage carrier state in *Salmonella typhimurium*
Abram Aertsen (Belgium)

12^{10} 10 • The evolution of social interactions in spatially organised bacterial communities
Sara Mitri (Great Britain)

12^{30}–13^{30} Industrial exhibition and lunch break

13^{30}–15^{25} **Sociomicrobiology and microbial community networks**
Chair Ines Mandic-Mulec (Slovenia)

13^{30} 11 • Social evolution of microbes
Kevin Foster (Great Britain)

14^{05} 12 • Less is more – bacterial gene loss results in division of labour and the formation of intercellular networks
Christian Kost (Germany)

14^{25} 13 • Social networking pays off
Polonca Štefanič (Slovenia)

14^{45} 14 • Bacterial sense of small – the role of volatiles as infochemicals in bacterial inter-specific interactions
Paolina Garbeva (Netherlands)

15^{05} 15 • Enhanced biofilm formation of bacterial consortia from various natural habitats suggests ubiquity of synergism in multispecies biofilms
Mette Burmølle (Denmark)

15^{25}–18^{30} **Poster Session I** (see page 18)

08^{30}–10^{05} **Microbial interactions with eukaryotic hosts I**
Chair Gabriele Berg (Austria)

08^{30} Keynote Lecture
16 • Exploiting the rhizosphere microbiome to improve crop production
Peter Bakker (Netherlands)

09^{05} 17 • *Sphagnum*-associated microbiome – Treasure chest or Pandora's box?
Anastasia Bragina (Austria)

09^{25} 18 • Stability of multispecies bacterial communities – signaling networks may stabilize microbiomes
Sándor Pongor (Italy)

09^{45} 19 • Ecology of bacteria associated with plant litter – decomposers, cheaters and mycophages
Petr Baldrian (Czech Republic)

10^{05}–10^{35} Industrial exhibition and coffee break

10^{35}–12^{10} **Microbial interactions with eukaryotic hosts II**
Chair Jan Dirk van Elsas (Netherlands)

10^{35} Keynote Lecture
20 • Are microbial communities the sum of their parts? Dismantling and reassembling the *Drosophila* gut microbiota
Peter Newell (United States)

11^{10} 21 • A spatial examination of the bacterial community diversity and lignocellulose composition of wood present in the gastrointestinal tract of *Panaque nigrolineatus*, a wood-eating catfish
Joy Watts (Great Britain)

11^{30} 22 • Insights into the bovine rumen plasmidome
Itzhak Mizrahi (Israel)

11^{50} 23 • Functional specificities of microorganisms on two different human skin body sites
Alban Mathieu (France)

12^{10}–13^{10} Industrial exhibition and lunch break

13^{10}–14^{45} **Microbial community diversity and ecosystem function I**
Chair James Prosser (Great Britain)

13^{10} Keynote Lecture
24 • The big picture – lessons from a rapidly expanding genomic tree of life
Phil Hugenholtz (Australia)

13^{45} 25 • Responses of soil bacterial and fungal communities to extreme soil drought and rewetting
Romain Barnard (France)

14^{05} 26 • Aggregate-scale spatial distribution of extracellular enzymes and microbial diversity in soil
Genevieve Grundmann (France)

14^{25} 27 • Bacterial laccases in genomes and metagenomes – from genes to functional enzymes
Luka Ausec (Slovenia)

14^{45}–15^{15} Industrial exhibition and coffee break

Scientific Programme • Tuesday, 11 June 2013

15^{15}–17^{00} **Microbial community diversity and ecosystem function II**
Chair Christoph Tebbe (Germany)

15^{15} Keynote Lecture
28 • Genome discovery from managed microbial communities
Timothy Vogel (France)

15^{50} 29 • Nutrient turnover and food web structures in soils with different histories of rice cultivation in China
Michael Schloter (Germany)

16^{10} 30 • Is richness important for the functional performance of microbial communities?
David Johnson (Switzerland)

16^{30} 31 • Reconstructing bacterial and viral genomes using metagenomics
Carolina Megumi Mizuno (Spain)

17^{00}–20^{00} **Poster Session II** (see page 26)

08^{30}–10^{05} **Beneficial microbes**
Chair Pascal Simonet (France)

08^{30} Keynote Lecture
32 • Approaching an ecosystem from the top-down and bottom-up – using multi omic approaches to investigate the gut
Julian Marchesi (Great Britain)

09^{05} 33 • Gastrointestinal behavior of *Butyricicoccus pullicaecorum*, a novel butyrate-producing isolate with probiotic potential in inflammatory bowel diseases
Annelies Geirnaert (Belgium)

09^{25} 34 • Bacterial chitinase with phytopathogen control capacity from suppressive soil isolated by functional metagenomics
Sara Sjöling (Sweden)

09^{45} 35 • Mining bacterial inter-specific interactions for discovering novel antimicrobial compounds
Olaf Tyc (Netherlands)

10^{05}–10^{25} Industrial exhibition and coffee break

10^{25}–12^{00} **Ecophysiology**
Chair Amalia Karagouni (Greece)

10^{25} Keynote Lecture
36 • The remarkable capacity of *Burkholderia* spp. to interact with soil fungi
Jan Dirk van Elsas (Netherlands)

11^{00} 37 • Growing on the nasty stuff – organohalide respiration unravelled by genomics and proteomics
Tobias Goris (Germany)

11^{20} 38 • Sequence-inferred microbial community interactions involving *Metallosphaera yellowstonensis* MK-1
Margaret Romine (United States)

11^{40} 39 • Genotype-phenotype relationship in denitrifying bacteria?
Åsa Frostegård (Norway)

12^{00}–12^{30} Industrial exhibition and coffee break

12^{30}–13^{45} **Round table discussion**
40 • The plant microbiome – new perspectives for biocontrol and growth promotion
Participants:
Gabriele Berg (Austria)
Jan Dirk van Elsas (Netherlands)
Lêda Mendonça Hagler (Brazil)
Dror Minz (Israel)
Henry Müller (Austria)
Kornelia Smalla (Germany)

14^{15}–18^{00} **Tour Postojna Caves** (see page 36)

from 19^{30} **Conference dinner at Ljubljana Castle** (see page 36)

Scientific Programme • Thursday, 13 June 2013

09^{00}–10^{35} Microbial responses to anthropogenic impacts and approaches to alleviate them I
Chair Timothy M. Vogel (France)

09^{00} Keynote Lecture
41 • When worlds collide – the biosphere meets the geosphere in heavy oil reservoirs
Ian Head (Great Britain)

09^{35} 42 • Identification of a plasmid-encoded novel amidase that catalyzes the first step in degradation of the groundwater pollutant 2,6-dichlorobenzamide (BAM) in *Aminobacter* sp. MSH1
Jeroen T 'Syen (Belgium)

09^{55} 43 • Mobilomic analysis suggests an important role of mobile genetic elements in bacterial adaptation towards pesticide degradation in on-farm biopurification systems
Vincent Dunon (Belgium)

10^{15} 44 • Functional gene responses in a perfect world – Can we trust quantification of transcripts in soil in response to man-made chemicals?
Carsten Suhr Jacobsen (Denmark)

10^{35}–11^{05} Industrial exhibition and coffee break

11^{05}–12^{40} **Microbial responses to anthropogenic impacts and approaches to alleviate them II**
Chair Elizabeth Wellington (Great Britain)

11^{05} 45 • Spread of tetracycline resistance genes from cow excrements to pasture soils
Martina Kyselkova (Czech Republic)

11^{25} 46 • Antibiotic resistance associated with waste water treatment plant effluent
Gregory Amos (Great Britain)

11^{45} 47 • *Escherichia coli* – a model for monitoring antibiotic resistance spread into the environment and quantification of resistance genes in wastewater treatment plants
Damiano Cacace (Germany)

12^{05} Keynote Lecture
48 • Advances in microbial ecology – drivers and limitations
James Prosser (Great Britain)

12^{40}–12^{50} **Farewell and announcement of BAGECO 13**

Poster Presentations • Monday, 10 June 2013

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Prokaryotic evolution and horizontal gene transfer

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Mark Liles (Auburn, ME, United States)

P 004 Tracking microbial evolution through CRISPRs
Laura Sanguino Casado (Ecully, France)

P 006 Comparative genome-analysis of a non-photosynthetic basal lineage of Cyanobacteria found in both dark and light habitats
Rochelle Soo (Brisbane, Australia)

P 008 Distribution and evolution of aromatic degrading genes in Alphaproteobacteria
Sajan Raju (Helsinki, Finland)

P 010 Genomic analysis of anaerobic haloalkaliphilic chitinolytic bacterium "Chitinivibrio alkaliphilus" Acht1, a first cultured representative of the candidate phylum TG3
Nikolay Ravin (Moscow, Russian Federation)

P 012 Inspecting genome histories in *A. tumefaciens* to reveal the role of adaptation in bacterial cladogenesis
Florent Lassalle (Villeurbanne, France)

P 014 Genome sequence of the arsenite oxidizing strain *Aliihoeflea* sp. 2WW
Lucia Cavalca (Milano, Italy)

P 016 Promotion of mutation by extracellular nucleic acids in *Escherichia coli*
Takehiko Kenzaka (Tondabayashi, Japan)

P 018 The capacity for multistability in anaerobic chaos – (eco)systems approach to bacterial and archaeal microbial assemblages in rice fields, animal rumen and biogas reactors
Blaž Stres (Domžale, Slovenia)

Sociomicrobiology and microbial community networks

P 020 Network analysis reveals the co-occurrence patterns of bacterial phylotypes in a long-term phytoremediation field experiment
Shinjini Mukherjee (Helsinki, Finland)

P 022 Mining chitinase-related sequences in mangrove sediments by metagenomics
Joelma Marcon (Piracicaba, Brazil)

P 024 Identification of gene functions involved in synergistic interactions in a pesticide degrading multispecies bacterial consortium
Pieter Albers (Leuven, Belgium)

P 026 *Bacillus subtilis* social interactions – different origin, same chat
Polonca Štefanič (Ljubljana, Slovenia)

P 028 Molecular and cultivation based approaches to identifying bacterial interspecies interactions in biological soil crusts
Ulisses Nunes Da Rocha (Berkeley, CA, United States)

Microbial interactions with eukaryotic hosts

P 030 Biomass hydrolyzing enzymes identified by functional screening of a metagenomic library from algal biofilms
Marjolaine Martin (Gembloux, Belgium)

P 032 Rapid and selective colonization of fungal hyphae by antifungal bacteria in the rhizosphere
Max-Bernhard Rudnick (Wageningen, Netherlands)

P 034 *Allolobophora hrabei* and its effects on soil microorganisms in steppe ecosystems
Dana Elhottová (České Budějovice, Czech Republic)

P 036 Opposite effects of two polyphenol oxidases of *Streptomyces* on plant growth promotion
Fung-Hsuan Kao (Taipei, Taiwan)

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Eric Dugat-Bony (Thiverval-Grignon, France)

P 040 The host microbiome and plant health – seed colonizing microbes from disease suppressive vermicompost alter zoospore chemotaxis, encystment and germination of *Pythium aphanidermatum*
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P 042 Host specificity of the plant growth-promoting cooperation between *Azospirillum* and rice
Benoît Drogue (Villeurbanne, France)

P 044 Warning – bacterial stink bomb! The potential of non cyanogenic *Pseudomonas* for biological control of *Phytophthora infestans*
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P 048 The diversity and biogeography of bacterial communities associated with pioneer plant species in the Arctic
Riitta Nissinen (Jyväskylä, Finland; Groningen, Netherlands)

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Phillip James (Coventry, Great Britain)

P 052 Comparison of two assays for the screening of phytogenics against *Lawsonia intracellularis*
Andreas Köstelbauer (Tulln, Austria)

P 054 Influence of *Bacillus amyloliquefaciens* FZB42 on the disease severity of bottom rot and the rhizosphere microbial community of field grown lettuce (*Lactuca sativa*)
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P 056 Phylogenetic clustering of *Bradyrhizobium* symbionts on host legume clades
Matthew Parker (Binghamton, NY, United States)

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Microbial community diversity and ecosystem function

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Karen De Roy (Gent, Belgium)

P 064 Genetic diversity of archaeal ammonia oxidizers drives potential nitrification rates in agricultural soils
Michele C Pereira E Silva (Groningen, Netherlands)

P 066 High rates of denitrification and nitrous oxide emission in biological soil crusts from an arid desert
Raeid Abed (Muscat, Oman)

P 068 Study of bacterial diversity in the topsoil and below the hardpan in an agricultural soil by metagenomics following by two analysis pipelines
Aurore Stroobants (Gembloux, Belgium)

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Fernando Dini Andreote (Piracicaba, Brazil)

P 072 Skewing the trophic structure of bacterial and fungal communities through sequential enrichments involved in lignocellulose and furanic compound bioconversion
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P 074 Understanding bacterial response to environmental perturbations at multiple spatial scales using a metagenomic approach
Jean-Sébastien Beaulne (Ecully, France)

P 076 Metatranscriptomics of microbial communities from mangroves
Armando Cavalcante Franco Dias (Piracicaba, Brazil)

P 078 Assessment of the effects of genetically modified (GM) maize cultivation on ammonia-oxidizing bacterial and archaeal communities
Simone Raposo Cotta (Piracicaba, Brazil)

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Stefan Pfeiffer (Tulln, Austria)

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David Kamanda Ngugi (Thuwal (Jeddah), Saudi Arabia)

P 084 Soil aggregates – protective hot spots for microbes against oxidation
Shamina Pathan (Florence, Italy)

P 086 Microbial biogeography in Arctic snowpacks
Catherine Larose (Ecully Cedex, France)

P 088 Microbial community gene expression of a cheese ecosystem through a metatranscriptomic analysis
Pascal Bonnarme (Thiverval-Grignon, France)

P 090 Diversity and physiology of *Trichoderma* associated with *Burkholderia* spp. in the nests of Borneo’s exploding ants
Lea Atanasova (Vienna, Austria)

P 092 Mapping the niche of archaeal ammonia-oxidizers in Icelandic grassland soils – a field study and microcosm approach
Anne Daebeler (Wageningen, Utrecht, Netherlands)

P 094 Small-scale variation in bacterial community structure and function within freshwater ponds
Gavin Lear (Auckland, New Zealand)

P 096 Characterization of *Agrobacterium tumefaciens* species complex isolates from agricultural soils of Slovenia
Janja Lamovšek (Ljubljana, Slovenia)

P 098 Do tree species influence community structure and richness of ammonia oxidizing bacteria at three temperate forest sites?
Sandrine Malchair (Liegé, Belgium)

P 100 Biogeography of soil *Burkholderia* populations
Nejc Stopnišek (Zurich, Switzerland)

P 102 Impact of long-term mineral fertilization inputs on composition of bacterial, fungal and AOB communities and on microbial enzymatic activities
Jiri Cuhel (Brno, Czech Republic)

P 104 Molecular analysis of microorganisms of deep subsurface thermal waters in Western Siberia
Vitaly Kadnikov (Moscow, Russian Federation)

P 106 Consequences of Amazon rainforest conversion – metagenomic analysis of soil-borne microbial community and their functional attributes
Lucas William Mendes (Piracicaba, Brazil; Wageningen, Netherlands)

P 108 A long-term artificial soil study – minerals and charcoal control the microbial response to spiked litter and phenanthrene
Doreen Babin (Braunschweig, Germany)

P 110 "High Resolution Melt" analysis – a novel method for fast screening of environmental samples for changes in bacterial phylogenetic composition prior to deep sequencing
Mathis Hjort Hjelmsø (Copenhagen, Denmark)

P 112 Microbial community composition in the traps and periphyton of two carnivorous *Utricularia* species
Jiri Barta (Ceske Budejovice, Czech Republic)

P 114 Pyrosequencing analysis of enrichment culture used to remove perchlorate in spent regenerant brine
Yeonghee Ahn (Busan, Republic of Korea)

P 116 Biodiversity of soil microorganisms in the Trindade Island/Brazil
Victor Pylro (Viçosa, Harpenden, Brazil)

P 118 Modeling of structure and Interactions the inorganic pyrophosphatase as from metagenomic sample
Gisele Rodrigues (Jaboticabal, Brazil)

P 120 Occurrence of bacterial laccase in Brazilian podzols
Elisa Matos (Piracicaba, Brazil)

P 122 Seasonal effects on the bacterial community in upper horizons of soil profile in deciduous forest
Rubén López-Mondéjar (Prague, Czech Republic)

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Stéphanie Roosa (Mons, Belgium)

P 126 Diversity of bacteria associated with natural vegetation growing in landfill of long-term PCB contaminated soil
Lucie Musilova (Prague, Czech Republic)

P 128 RNA and DNA-based profiling of soil microbial communities in conventional, innovative low-pesticide input and organic agricultural systems
Emilie Laroche-Ajzenberg (Mont-Saint-Aignan, France)

P 130 Quantifying cellulolytic bacteria in the rumen of sheep under a diet with sugarcane bagasse
Emiliana Romagnoli (Piracicaba, Brazil)

P 132 Spatial and seasonal variation of the rhizospheric bacterial community of two legumes of Brazilian semiarid uncovered by large-scale sequencing by Ion Torrent
Rodrigo Taketani (Jaguariuna, Brazil)

P 134 Soil quality and microbial community changes after a decade of different tillage at two Slovenian sites with different pedo-climatic conditions
Anela Kaurin (Ljubljana, Slovenia)

P 136 Structure of bacterial communities of *Vigna unguiculata* (L.) Walp. rhizosphere soils in dark soil from Amazon
Rosineide Cardoso (Manaus, Brazil)

P 138 Combination of stable isotope probing and metatranscriptomics to examine the activity of aerobic methanotrophs in lake sediment
Marc Dumont (Marburg, Germany)

P 140 Efficiency of three extraction methods of PCR amplifiable DNA from protozoa
Susana Santos (Roskilde, Denmark)

P 142 Microbial diversity and functional annotation of sugar-cane cultivated soil by next-generation sequencing approach
Eliamar Aparecida Nascimbém Pedrinho (Jaboticabal, Brazil)

P 144 Metagenomic analysis of arctic marine microbial communities
Lea Benedicte Skov Hansen (Copenhagen, Denmark)

P 146 Rhizosphere and bulk soil microbial communities affected by soil type but not by biocontrol strain *Pseudomonas jessenii* RU47
Susanne Schreiter (Braunschweig, Germany)

P 148 Prokaryotic diversity of wetland evaluated both by isolation and cultivation-independent analyses
Chi Nam Seong (Suncheon, Republic of Korea)

P 150 Changes in microbial communities in alpine soil exposed to increased nitrogen and ozone levels
Salome Schneider (Uppsala, Sweden; Zurich, Switzerland)

P 152 Exploiting and bioprospecting Brazilian biomes for phosphorus disponibilization
Fernando Dini Andreote (Piracicaba, Brazil)

P 154 Efficacy of ozonized water for microbial decontamination of fruits
Salama Ouf (Almadinah Almunawwarah, Saudi Arabia)

P 156 Bacterial diversity and soil physiology dynamics in no-till agriculture soils under different managements
Luis Wall (Bernal, Argentina)

Beneficial microbes

P 158 The probiotic potentials of some lactic acid bacteria from some African fermented foods
Hope Okereke (Uturu, Nigeria)

P 160 Evaluation of bacterial nanocellulose membranes – activity and stability study of the antimicrobial nisin peptide
Adalberto Pessoa (São Paulo, Brazil)

P 162 Gut microbiota, host energy metabolism, and potential probiotic effects
Evelyn Hackl (Tulln, Austria)

P 164 Bacteria isolated from the deep-sea hydrothermal field of Kolumbo submarine volcano – a potential source of new bioactive compounds
Maria Bourbouli (Athens, Greece)

P 166 Oil-degrading rhizosphere and endophytic actinomycetes to enhance the phytoremediation ability of corn plants in oil-polluted soil in the United Arab Emirates
Khaled El-Tarabily (Al-Ain, United Arab Emirates)

P 168 Ecology of *Lysobacter* species in natural disease suppressive soils
Ruth Gomez Exposito (Wageningen, Netherlands)

P 170 Genomic characterization of pilus-deficient derivatives of *Lactobacillus rhamnosus* GG
Pia Rasinkangas (Helsinki, Finland)

P 172 Antagonistic effects of the indigenous Greek isolate *Streptomyces rochei* ACTA1551, on *Fusarium oxysporum* f.sp. *lycopersici*
Grammatiki Kanini (Zografou, Greece)

Ecophysiology

P 174 Desert rhizosphere microbiota and plant resistance to water stress
Sara Borin (Milan, Italy)

P 176 Bet-hedging as a fitness character in *Paracoccus denitrificans* during transitions from aerobic respiration to denitrification
Linda Bergaust (Ås, Norway)

P 178 Differential molecular-based monitoring of the filamentous growth of *Sphaerotilus natans*
Jean-Jacques Pernelle (Antony Cedex, France)

P 180 Prodigiosin – mechanisms of antimicrobial action
Tjaša Danevčič (Ljubljana, Slovenia)

Microbial responses to anthropogenic impacts and approaches to alleviate them

P 182 Biodegradation of diesel oil using gene cloned *alkB* obtained from a library metagenomics encoding the enzyme alkane hydroxylase
Silvana Pompeia Val-Moraes (Jaboticabal, Brazil)

P 184 Impact of amendment with raw organic wastes on the prevalence of human pathogens and antibiotic resistance spread in agricultural fields
Benjamin Youenou (Villeurbanne, France)

P 186 Examining *Acidobacteria* as bio-indicators in Amazon soils – an approach using landscape-scale
Acácio Aparecido Navarrete (Piracicaba, Brazil)

P 188 Draft genome of a MCPA-degrading *Sphingomonas* reveals unique genetic context of the less-studied *cadAB* genes in a transposon situated on a conjugative plasmid
Tue Kjærgaard Nielsen (Copenhagen, Denmark)

P 190 Management of arable soil to reduce eutrophication in changing climate – how no-till affects microbial communities and individual phylotypes
Timo Petteri Sipilä (Helsinki, Finland)

P 192 Linking soil functioning with functional microbial diversity in agricultural soils – the normal operating range of arginine degradation across the Netherlands
Agnieszka Szturc-Koetsier (Utrecht, Netherlands)

P 194 Tropical soil multi-contamination – a metagenomics approach to evaluate nickel influence in petroleum biodegradation
Natalia Taketani (Rio de Jeneiro, Brazil)

P 196 Emergence and persistence of resistance genes in sewage canalization networks of metropolitan areas: unravelling the relationship between antibiotic selective pressure and antibiotic resistance genes
Serena Caucci (Dresden, Germany)

P 198 Evaluation of the microbial water quality and the effects of rainfall at recreational waters and sand, in Athens, Greece
George Paraschas (Athens, Greece)

P 200 Change of the microbial community composition in geothermally used fluids due to plant operation dependent temperature alterations and operation failures
Anke Westphal (Potsdam, Germany)

P 202 Seasonal changes in bacterial and viral population in a waste water plant effluent
Maja Rupnik (Maribor, Ljubljana, Slovenia)

Free topics

P 204 Mutant from *Rhizobium tropici* SEMIA 4077, a microorganism which produces an exopolysaccharide of biotechnological relevance
Eliana Gertrudes De Macedo Lemos (Jaboticabal, Brazil)

P 206 Functional screening of a winter and a spring genomic DNA libraries obtained from soils in a winter wheat crop
Aurore Stroobants (Gembloux, Belgium)

P 208 Systems biology approaches predict inorganic N regulation of *xylM* and *xylE* gene expression and xylene degradation by *Pseudomonas putida* mt-2 in soil microcosms
Nanna Svenningsen (Copenhagen, Denmark)

P 210 Analysis of metagenomic sequences from Brazilian mangrove sediments with potential for enzymatic activities
Júlia Ronzella Ottoni (Paulinia, Brazil)

P 212 DNA sorption blocker "G2" increase DNA recovery from subsoil clay sediment >1.000 times
Tue Kjærgaard Nielsen (Copenhagen, Denmark)

P 214 Pan genome analysis of *Lactobacillus crispatus*
Teija Ojala (Helsinki, Finland)

P 216 Abundance of *mcrA* and CH_4 emissions from different vinasse distribution systems in a Brazilian sugarcane mill
Bruna Oliveira (Piracicaba, Brazil)

P 218 Quantification of antibiotic resistance gene input and output in wastewater treatment plant
Antti Karkman (Helsinki, Finland)

P 220 Implications of interaction between human pathogen *Campylobacter jejuni* and its bacteriophages
Nika Janez (Solkan, Slovenia)

P 222 Ammonia concentration as the major driver of niche specialisation between AOA and AOB
Cecile Thion (Aberdeen, Great Britain)

P 224 Characterization of *Chromobacterium violaceum* alkaline phosphatase coding genes, *phoA1* and *phoA2*
Fernanda Vasconcelos (São Paulo, Brazil)

P 226 Licensing strategies – the basic concern is to achieve the best quality of patent application
Levin Pal (Ljubjana, Slovenia)

P 228 LuxS-dependent quorum-sensing affects mutation rate plasticity in *Escherichia coli*
Rok Krašovec (Manchester, Great Britain)

P 230 Mixed target genetic screening of a fosmid metagenomic soil library
Samuel Jacquiod (Ecully, France)

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Prokaryotic evolution and horizontal gene transfer

P 001 Transcriptional noise in natural and synthetic *E. coli* promoters
Luise Wolf (Basel, Switzerland)

P 003 Assessing the permissiveness of complex bacterial communities towards conjugal plasmids – Development of a novel method
Uli Klümper (Kgs. Lyngby, Denmark)

P 005 Lysogeny mediates the survival of *Escherichia coli* in marine sediment
Stanley Lau (Kowloon, Hong Kong, China)

P 007 saAFLP and hin-region – new approaches for biodiversity and taxonomy investigation, and diagnostics of the genus *Xanthomonas*
Nataliya Punina (Moscow, Russian Federation)

P 009 Genomics of a unintrogressed *Campylobacter coli* clade 3 strain
Astrid De Haan (Helsinki, Finland)

P 011 Finding specific ecological adaptations of bacterial species – a quest for a renewed taxonomic description of bacteria
Xavier Nesme (Villeurbanne, France)

P 013 *Aeromonas* spp. – Ubiquitous or specialized bugs?
Maria Elena Martino (Legnaro, Italy)

P 015 Replicon-dependent bacterial genome evolution in *Sinorhizobium meliloti*
Marco Bazzicalupo (Sesto Fiorentino, Firenze, Italy)

P 017 The use of microfluidic devices to understand how different transcriptional responses to external stimuli evolve in natural isolates of *Escherichia coli*
Matthias Kaiser (Basel, Switzerland)

Sociomicrobiology and microbial community networks

P 019 Transcriptomics of quorum-sensing pathway revealed that transfers of the virulence Ti-plasmid and companion At-plasmid are co-regulated in *Agrobacterium tumefaciens*
Julien Lang (Gif-sur-Yvette, France)

P 021 Structure and sociality of the lettuce core microbiome
Massimiliano Cardinale (Graz, Austria)

P 023 Systematic molecular measurements reveal key microbial populations driving community-wide phenotype
Emilie Muller (Esch sur Alzette, Luxembourg)

P 025 Crime and punishment of social interactions in *Bacillus subtilis*
Anna Oslizlo (Ljubljana, Slovenia)

P 027 Genetic and phenotypic diversity of *Bacillus subtilis* isolates obtained from a tomato rhizosphere
Polonca Stefanic (Ljubljana, Slovenia)

Microbial interactions with eukaryotic hosts

P 029 Methanotrophs and methanogens in the rice endophyte microbiome
Angela Sessitsch (Tulln, Austria)

P 031 Sheep rumen microbiome sequencing using Ion Torrent (PGM) platform
Rodrigo Mendes (Jaguariuna, Brazil)

P 033 Meta-transcriptomics of the rhizosphere microbiome – the quest for bacterial genera and traits involved in natural plant protection
Emilie Chapelle (Wageningen, Netherlands)

P 035 How do the polyphenol oxidases of *Streptomyces* affect plant growth promotion?
Han-Yu Yang (Taipei, Taiwan)

P 037 *Metarhizobium medetiranium* and *Metarhizobium tianshanense* – symbionts of sainfoin
Sofiya Hapchaeva (Moscow, Russian Federation)

P 039 ECF sigma factors – how endophytes sense the plant
Raheleh Sheibani Tezerji (Tulln, Austria)

P 041 Two-component signalling systems in the *Azospirillum* – from comparative genomics to functional gene analysis
Stephanie Borland (Villeurbanne, France)

P 043 Genome and transcriptome analysis reveals mechanisms involved in beneficial plant-microbe interaction of the stress protecting agent *Stenotrophomonas rhizophila* DSM14405T
Henry Mueller (Graz, Austria)

P 045 Comparative analysis of different *Burkholderia* genomes reveals the basis of fungal interactive bacterial strategies
Rashid Nazir (Groningen, Netherlands)

P 047 The ecology of microbial communities associated with the traps of aquatic carnivorous *Utricularia* species
Dagmara Sirova (Ceske Budejovice, Czech Republic)

P 049 Behavior in vitro of rumen ecosystem using different grazing agroecosystems
Niurca Gonzalez (La Habana, Cuba)

P 051 Diversity of endophytes fungi associated to two different medicinal plants
Claudia Souza Ramalho Sánchez (Mogi das cruzes, Brazil)

P 053 Tackling the specificity of the marine sponge microbiome – a biogeographical approach
M. Asunción Lago-Lestón (Faro, Portugal)

P 055 Assessment of the microbial community in the cathode compartment of a plant microbial fuel cell
Michael Rothballer (Munich, Neuherberg, Germany)

P 057 Metagenome-sequencing of the obligate symbiotic *Mycoplasma*-related endobacteria of arbuscular mycorrhizal fungi
Gloria Torres-Cortés (Munich, Germany)

P 059 Exploring the bovine rumen bacterial community from birth to adulthood
Itzhak Mizrahi (Tel Aviv, Israel)

Microbial community diversity and ecosystem function

P 061 Characterization of a novel strain of the genus *Actinopolyspora*, an extremely halophilic actinomycete isolated from Saudi Arabia
Manal Kiki (Jeddah, Saudi Arabia)

P 063 Microbial community structure and functional potential associated with four boreal vegetation types
Mary-Catherine Lewis (Fairbanks, United States)

P 065 Loss in microbial diversity affects nitrogen cycling in soil
Aymé Spor (Dijon, France)

P 067 Sequence capture – a new tool for metagenomics
Denis Le Paslier (Evry, France)

P 069 16S rRNA gene variability in bacterial genomes and its consequences for bacterial community analyses
Tomáš Větrovský (Prague, Czech Republic)

P 071 Analysis of bacterial communities in Lake Kivu by comparative DNA- and RNA-based pyrosequencing
Özgül Inceoglu (Brussels, Belgium)

P 073 Using a metagenomic approach to explore microbial community structure and function in Arctic snow
Lorrie Maccario (Ecully, France)

P 075 Determination of soil microbial community structure and respiration during soil cooling
Marc Auffret (Aberdeen, Great Britain)

P 077 Metagenomic discovery of rhizosphere microbiome dynamics from mandacaru in Caatinga
Clederson Ferreira (Jaguariuna, Brazil)

P 079 Survival and adaptation of introduced phosphate-solubilizing bacteria (PSB), *Bacillus* sp. during phytoextraction of Cd-contaminated soil
Hee Sun Moon (Daejeon, Republic of Korea)

P 081 Diversity and evolution of the microbial communities at the rhizosphere of *Quercus ilex* subsp. *ballota* after a wildfire
Manuel Fernández-López (Granada, Spain)

P 083 The white rot fungus *Phanerochaete chrysosporium* structures the diversity of the associated bacterial community during wood decay
Vincent Herve (Champenoux, France)

P 085 Bacterial succession in a developing salt marsh ecosystem
Francisco Dini-Andreote (Groningen, Netherlands)

P 087 Metaproteogenomic insights of contaminated microbial communities in marine and freshwater environments
David Gillan (Mons, Belgium)

P 089 Microbial characterization of drinking water in Lithuania
Daiva Staradumskytė (Kaunas, Lithuania)

P 091 Total microbial activity and microbial composition of a mangrove sediment are reduced by oil pollution at a site in the Arabian Gulf
Khaled El-Tarabily (Al-Ain, United Arab Emirates)

P 093 Evidence for remarkable genetic plasticity of bacterial linear plasmids from extreme environments
Julian Rafael Dib (Münster, Germany)

P 095 How to make the most of agro-ecosystems – the impact of agricultural practice on microbial diversity and sulfur cycling
Mirja Guldner (Sydney, Australia)

P 097 Is everything everywhere? – Metagenomic analysis of Arctic and Antarctic marine bacteria communities
Søren Sørensen (Copenhagen, Denmark)

P 099 Thaumarchaeota are pioneer organisms in acidic volcanic soils from South of Chile
Marcela Hernandez (Marburg, Germany)

P 101 Effect of stratification on bacterial communities in forest soil and isolation of key bacterial taxa
Salvador Lladó (Prague, Czech Republic)

P 103 Drivers of ammonium oxidizing communities in soils from sugarcane fields in Brazil
Júlia Lima (Piracicaba, Brazil)

P 105 Microbial ecology of high-temperature springs of Caldera Uzon, Kamchatka
Vadim Gumerov (Moscow, Russian Federation)

P 107 Effects of aromatic hydrocarbons addition in "Amazon Dark Earth" on the community and abundance of total bacteria and the catabolic gene *bph*
Maria Julia Brossi (Piracicaba, Brazil)

P 109 Distribution of degradation activities of woody materials in marine eukaryotes, thraustochytrids
Yousuke Taoka (Miyazaki, Japan)

P 111 Effects of gentle remediation options (GRO) on the bacterial community structure of trace metal-contaminated soils
María Touceda-González (Santiago de Compostela, Spain)

P 113 Endophytic bacterial communities from medicinal plants – a new source of bioactive compounds producing isolates
Giovanni Emiliani (Florence, Italy)

P 115 Impact of a genetic modification on bacterial diversity in the rhizosphere of maize across Europe
Astrid Näther (Braunschweig, Germany)

P 117 Abundance and diversity of clostridia in biogas production plants
Anja B. Dohrmann (Braunschweig, Germany)

P 119 Reduction in bacterial functioning mirrors reduction in total and functional diversity after a strong soil disturbance
Heike Schmitt (Wageningen, Netherlands)

P 121 Subspecies diversity of the species *Novosphingobium acidiphilum* in Lake Grosse Fuchskuhle
Stefanie P. Glaeser (Giessen, Germany)

P 123 Metagenomic discovery of rhizosphere microbiome dynamics from mandacaru in Caatinga
Clederson Ferreira (Jaguariuna, SP., Brazil)

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P 125 Fungal-bacterial networks – an overseen potential to overcome limitations for effective contaminant degradation in vadose soils
Julia Giebler (Leipzig, Germany)

P 127 Symbiotic diazotrophic diversity and nitrogen fixation in the Brazilian Atlantic Forest
Alice Cassetari (Piracicaba, Brazil)

P 129 Metagenomic insights into the rhizosphere of semiarid soils – functional conservation and phylogenetic variability in a drought mediated environment
Rodrigo Taketani (Jaguariuna, Brazil)

P 131 A bacterial ecology study to improve shelf life of food products – meat microflora in swine fed with polyphenols from olive mill waste
Lisa Carraro (Legnaro, Italy)

P 133 Bacterial diversity of sugarcane cultivated soil comparing v3 region from 16S rRNA
Wellington Marcelo Queixas Moreira (Jaboticabal, Brazil)

P 135 Culturable actinobacterial diversity from a Brazilian restored forest fragment
Tiago Zucchi (Jaguariúna, Brazil)

P 137 Functional microbial community shifts due to bacterial invasion revealed by community level physiological profiling in a soil dilution-to-extinction experiment
Cyrus Mallon (Groningen, Netherlands)

P 139 Abundance and community structure responses of sulphur oxidizing bacteria during elemental sulphur oxidation in soil microcosms
Maria Tourna (Hamilton, New Zealand)

P 141 Exploring glyphosate-induced effects on turnover of root biomass and on rhizosphere microbial community dynamics in plant-soil mesocosms
Valentina Imparato (Roskilde, Denmark)

P 143 Diversity and structure of the soil microbiome under long-term organic and conventional farming
Martin Hartmann (Zurich, Birmensdorf, Switzerland)

P 145 Effects of rainfall events on microbial processes in forest soil
Jaroslav Šnajdr (Praha, Czech Republic)

P 147 Application of biocontrol strain *Pseudomonas jessenii* RU47 did not impact the fungal community in three different bulk soils
Susanne Schreiter (Braunschweig, Germany)

P 149 Influence of pharmaceuticals on composition of 16S rRNA and bacterial laccases genes in wastewater treatment bioreactors
Vesna Jerman (Ljubljana, Slovenia)

P 151 Resistance and resilience of the forest soil microbiome to soil compaction after logging operations
Martin Hartmann (Zurich, Birmensdorf, Switzerland)

P 153 List and ecological information of bacteria with valid names, isolated from Republic of Korea
Chi Nam Seong (Suncheon, Republic of Korea)

P 155 Microbiology of cave sediments – Is oligotrophy all that matters?
Blaž Stres (Domžale, Slovenia)

P 157 Microbial diversity and transformation of organic pollutants on distinct soil particle surfaces
Christoph Tebbe (Braunschweig, Germany)

Beneficial microbes

P 159 The indoor microbiome – Diversity and its control by beneficials?
Gabriele Berg (Graz, Austria)

P 161 Exploitation of PGPB and soil amendments in the phytoremediation of heavy metal contaminated sites
Vivek Balakrishnan Ravindran (Tulln, Austria)

P 163 Comparative genomics and antimicrobial activity of *Lysobacter* species
Irene De Bruijn (Wageningen, Netherlands)

P 165 Microalgae from Greek environments – potential use for biodiesel production
Konstantina Moysi (Athens, Greece)

P 167 Genome-based optimization of the biocontrol performance of the sugar beet endophyte *Pseudomonas poae* RE*1-1-14
Christin Zachow (Graz, Austria)

P 169 Analysis of acetic acid bacterial population during submerged industrial red wine vinegar production based on 16S-23S rDNA ITS regions
Darija Copot (Maribor, Slovenia)

P 171 Cellulolytic bacteria from Steinhouse Lake in Antarctic Peninsula
Itamar Melo (Jaguariúna, Brazil)

P 173 The industrial use of coding sequences from metagenomic expression libraries – lessons learned from variance partitioning, genetic constraints and sampling probabilities
Blaž Stres (Domžale, Slovenia)

Ecophysiology

P 175 Sensitivity of CH^4- and CO^2-producing microbial communities in anoxic peat to changing environment in a peatland transplantation experiment
Heli Juottonen (Helsinki, Finland)

P 177 Ecophysiology of *Vibrio harveyi* and *Vibrio ruber* in viscous media
Maja Borić (Ljubljana, Slovenia)

P 179 Exopolymer diversity and the role of levan in *Bacillus subtilis* biofilms
Iztok Dogša (Ljubljana, Slovenia)

Microbial responses to anthropogenic impacts and approaches to alleviate them

P 181 Amazonian resistome – evaluating antibiotic resistance abundance and diversity across a French Guiana forest soil gradient through metagenomic approaches
Joseph Nesme (Ecully, France)

P 183 Response of the soil microbial community to application of manure from difloxacin-treated pigs
Sven Jechalke (Braunschweig, Germany)

P 185 Perspectives on combinatorial biological, chemical and physical parameters in soil quality – an approach for sugarcane production systems
Acácio Aparecido Navarrete (Piracicaba, Brazil)

P 187 Chemically-enhanced microbial degradation of recalcitrant chlorinated compounds
Sebastien Cecillon (Ecully, France)

P 189 Detection of antibiotic resistance genes and mobile genetic elements in fermentation residues from biogas plants and manures from different pig producing facilities
Birgit Wolters (Braunschweig, Germany)

P 191 Response of methane cycling communities to the succession of re-vegetated cut-away peatlands
Anuliina Putkinen (Vantaa, Finland)

P 193 Relative impacts of cow faeces and antibiotics on microbial community of grassland soils with different history of management
Dana Elhottová (České Budějovice, Czech Republic)

P 195 Tic and metabolic analysis of the carbofuran degradation pathway in *Sphingomonas* sp. KN65.2
Oahn Nguyen (Heverlee, Belgium)

P 197 Dynamics of chlorophenol-degrading sphingomonads *in situ* by molecular and cultivation-based approaches
Anu Mikkonen (Lahti, Finland)

P 199 Influence of diclofenac on activated sludge microbial communities
Barbara Kraigher (Ljubljana, Slovenia)

P 201 Directed evolution of radiation-resistant acidophiles
Rok Tkavc (Bethesda, CA, United States)

Free topics

P 203 Micro fluid segment technique – application in microtoxicology and potential for investigation of interaction of microorganisms
Jialan Cao (Ilmenau, Germany)

P 205 Topoisomerase IV is required for circular chromosomes but not linear chromosomes in *Streptomyces*
Carton Chen (Taipei, Taiwan)

P 207 Correct OTUs – survey of different denoising and OTU clustering tools for amplicon sequence data
Kaisa Koskinen (Helsinki, Finland)

P 209 Understanding the catalytic mechanism for (p)ppGpp synthesis by Rel proteins
Balaji Prakash (Kanpur, India)

P 211 Characterization of new bacterial glycoside hydrolases isolated from agricultural soils using a functional metagenomic approach
Sophie Biver (Gembloux, Belgium)

P 213 Effects of static magnetic field on model wastewater bacteria and on ammonium removal from wastewater
Jasmina Filipič (Ljubljana, Slovenia)

P 215 Nitrogen amendment affects abundance and diversity of the *alkB*-harbouring indigenous microbial community in soil contaminated by petroleum hydrocarbons
Anja Grønskov (Frederiksberg, Denmark)

P 217 Comparative genomics of the meat spoilage bacteria *Leuconostoc gelidum* and *Leuconostoc gasicomitatum*
Riitta Rahkila (Helsinki, Finland)

P 219 Allies and foes – community ecology of bacteria and their bacteriophages in plant diseases of orchids
Tanja Dreo (Ljubljana, Solkan, Slovenia)

P 221 Polyphasic taxonomy of actinomycetes isolated from marine environment – Multilocus Sequence Analysis (MLSA) x DNA-DNA hybridization
Claudia Beatriz Menezes (Paulinia, Brazil)

P 223 Dermocosmetics – microbial diversity in raw materials and finished product
Anastasia-Aikaterini Delliou (Zografou, Greece)

P 225 Isolation and characterization of a heterologously expressed bacterial laccase from *Geobacter metallireducens*, a strict anaerobe
Marko Verce (Ljubljana, Slovenia)

P 227 Characterisation of *Escherichia coli* enterohemorrhagic O157:h7 in frozen bovine meat in Algeria
Barka Mohammed Salih (Tlemcen, Algeria)

P 229 Metagenome and metatranscriptome analyses of root-associated bacterial communities
Dror Minz (Bet Dagan, Israel)

Addendum for session on Microbial community diversity and ecosystem function

P 231 ODoSE – a webserver for genome-wide calculation of adaptive divergence in prokaryotes
Michiel Vos (Penryn, Great Britain)

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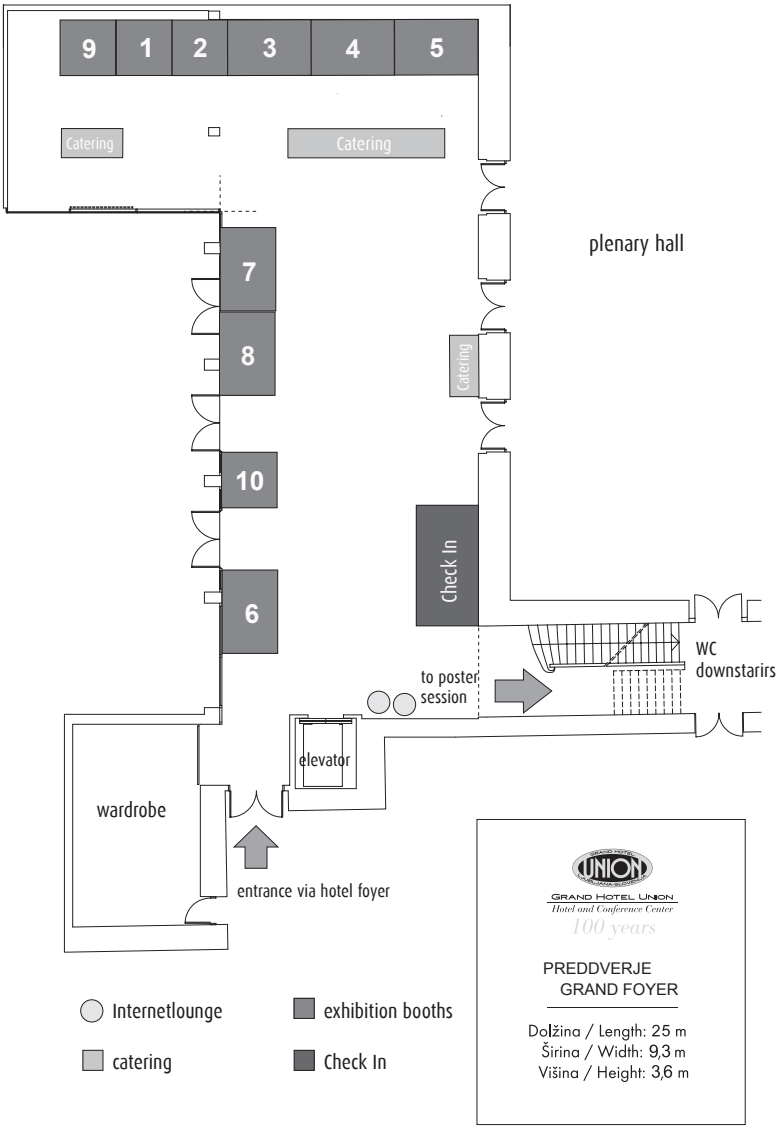
Plan of Exhibition Area



	Booth
CAMECA (Gennevilliers, France)	8
Carl Zeiss (Ljubljana, Slovenia)	5
Eurofins MWG Operon (Ebersberg, Germany)	3
Grga & Melita (Jastrebarsko, Croatia)	6
Mediline (Kamnik, Slovenia)	2
Mettler-Toledo (Ljubljana, Slovenia)	1
MO BIO Laboratories (Carlsbad, CA, United States)	7
MP Biomedicals (Illkirch, France)	4
Roche Diagnostics (Ljubljana, Slovenia)	10
Sekvenator (Ljubljana, Slovenia)	9

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Social and Cultural Programme

Welcome reception at Ljubljana town hall

Our Welcome Reception of BAGECO 12 will take place at the Ljubljana Town Hall. You are invited to join your colleagues and friends to tune into our 12th Symposium on Bacterial Genetics and Ecology. In this context the mayor of Ljubljana Mr. Zoran Janković would like to welcome you personally to his city. Some snacks and drinks will be provided.

© B. Kladnik

Date	9 June 2013
Begin	from 19^{00} hrs
Price	included

Tour Postojna Caves

We would like to invite you to our bus tour to the world-famous Postojna Caves, located southwest of Ljubljana. The caves are the greatest tourist attraction in Slovenia and also one of the world's largest karst monuments. These are the only caves in the world with a double track railway that will take you, on a special train, for a unique and adventurous ride into the cave, under spectacular underground arches, embellished with chandelier look-alike stalactites, and through a beautiful subterranean world full of playful limestone sculptures.

© postojnska-jama.eu

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Date	12 June 2013
Departure	14^{15} hrs
Return	18^{00} hrs
Price	20 EUR

Buses will be leaving from the main entrance of the Grand Hotel Union Executive to Postojna Caves. Your guided tour will start at 15^{30} and last approx. 90 minutes. Each participant will also receive a small lunch box.

Conference dinner at Ljubljana Castle

Our conference dinner will take you to the ancient castle of Ljubljana, which provides you with a spectacular view over the city. The enchanting atmosphere combined with great music will give you the chance to end this evening dancing and reflecting on high-quality BAGECO sessions.

© Tomo Jesenicnik

Date	12 June 2013
Begin	19^{30} hrs
Price	50 EUR

The Ljubljana Castle is within walking distance of the GHU Executive. Please follow our map on page 7 and either walk up to the castle or use your funicular tickets (each ticket is one way).

Recommendation: As the funicular only transports 30 people at a time and therefore to avoid long waiting lines, please try to arrive between 19^{00}–19^{30} hrs at the lower funicular station.
Please note that for the return trip the last funicular leaves at 24^{00} hrs. As you are very welcome to stay longer, please feel free to either walk back to the hotel or order yourself a cab (+386 31 23 40 00 or +386 40 88 77 66) at your own expenses (approx 5 EUR per ride).

Abstracts and Index of Abstract Authors

01

On becoming a symbiont – comparative genomic analyses of chemosynthetic symbionts of hydrothermal vent mussels and their closest free-living relatives

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The common perception of beneficial associations between animals and bacterial endosymbionts is that these have evolved only rarely from a few bacterial lineages that were uniquely adapted to a symbiotic lifestyle. Our recent analyses of the phylogenetic diversity of intracellular symbioses between chemosynthetic bacteria and deep-sea bathymodiolin mussels from hot vents, cold seeps and other chemosynthetic environments indicate that bathymodiolin mussels were colonized multiple times by many different lineages of bacteria (Petersen et al. 2012. Biol Bull 223: 123-137). At least four different lineages of free-living sulfur-oxidizing bacteria and six different lineages of free-living methane-oxidizing bacteria have established symbioses with bathymodiolin mussels, indicating that it may be evolutionarily "easy" to become an intracellular symbiont. To better understand the genetic attributes that enable chemosynthetic bacteria to adapt to a symbiotic lifestyle, we are currently comparing the genomes of the sulfur-oxidizing symbionts of bathymodiolin mussels with their closest free-living relatives, ubiquitous pelagic sulfur-oxidizers called SUP05 that dominate oxygen minimum zones worldwide, and have also been found in hydrothermal vent plumes (Anantharaman et al. 2013. PNAS 110: 330-335). Our analyses show that the *Bathymodiolus* symbiont has undergone massive rearrangements, and that as much as 38% of its genes are potentially of foreign origin. Genes and regions known to play a role in DNA transfer such as transposases and integron insertion sites are present in the *Bathymodiolus* symbiont and SUP05 bacteria, revealing a possible mechanism for horizontal gene transfer into these lineages. Genes of potentially foreign origin in the *Bathymodiolus* sulfur-oxidizing symbiont include genes for the use of hydrogen as an energy source (Petersen et al. 2011. Nature 476: 176-180) and a highly diverse array of toxins, which could be used for beneficial interactions with the host. The acquisition of these genes may have played a key role in enabling free-living sulfur-oxidizing bacteria to establish symbioses with deep-sea mussels and make optimal use of the energy sources available in their environment.

02

Trends and barriers to lateral gene transfer during microbial evolution

T. Dagan[1]
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(i) Trends and barriers to lateral gene transfer in prokaryotes Gene acquisition by lateral gene transfer (LGT) is an important mechanism for natural variation among prokaryotes. Laboratory experiments show that protein-coding genes can be laterally transferred extremely fast among microbial cells, inherited to most of their descendants, and adapt to a new regulatory regime within a short time. Recent advance in the phylogenetic analysis of microbial genomes using networks approach reveals a substantial impact of LGT during microbial genome evolution. Phylogenomic networks of LGT among prokaryotes reconstructed from completely sequenced genomes uncover barriers to LGT in multiple levels including (i) barriers to gene acquisition in nature including physical barriers for gene transfer between cells, (ii) genomic barriers for the integration of acquired DNA, and (iii) functional barriers for the acquisition of new genes.

03

Biofilms and mobile genetic elements – when the agenda of the mobile gene pool is to socialize

J. S. Madsen[1], M. Burmølle[1], L. Riber[1], L. H. Hansen[1], S. Sørensen[1]
[1]University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen, Denmark

Plasmids and other mobile genetic elements are found ubiquitously in bacterial genomes. Current research suggests a general interconnection between plasmid biology and biofilm ecology, which may in large part be explained through the fact that plasmids differ from the chromosomal elements of the genome because they are independent replicons that may petition their own evolutionary strategy. We aim to better the understanding of how, and to what extend, the mobile gene pool influences the behavior of bacteria. This can help reveal molecular mechanisms that influence the social evolution and the emergence of virulence factors in bacteria. We present the analysis of a number of recently isolated and sequenced plasmids, and reveal some of the molecular mechanisms and social predicaments behind plasmid induced biofilm formation. Several of these plasmids carry genes that initiate biofilm formation through enhanced attachment via fimbriae. We show that regulatory mechanisms normally coupled with such fimbriae, which are encoded on bacterial chromosomes (specifically associated with the c-di-GMP pool), are bypassed when encoded on plasmids. We propose that the plasmid-encoded fimbriae mediate surface and cell-cell interaction, whilst the host remains motile. This way, the plasmid dictates a "bifacial" phenotype of its host providing optimal conditions for successful dispersal of the plasmid by horizontal transfer. We further show that some of these plasmids, and many others, encode proteins that influence c-di-GMP levels, and regulate the behavior of the host via this innate system, which directs the transition between a biofilm and planktonic state. Our data indicates that genes associated with the response and/or regulation of c-di-GMP are widely found encoded on mobile genetic elements and are functional

04

Incorporating signatures of gene evolutionary history into phyletic profiling for large-scale inference of microbial gene function

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[2]Rudjer Boskovic Institute, Zagreb, Spain
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Introduction: Thousands of microbial genomes are available, yet even for the model organisms many genes have unknown function. Phyletic profiling is a technique that predicts gene function by comparing the presence/absence profiles of homologs across genomes. In addition, prokaryotic genomes contain an evolutionary signature of gene expression levels in the

codon usage biases, where highly expressed genes prefer the codons adapted to the cellular tRNA pools.

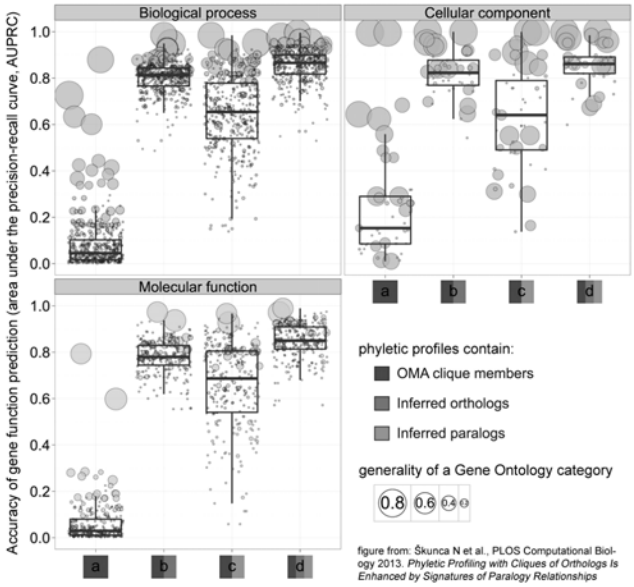
Objectives: We aimed to augment the existing phyletic profiling approaches by incorporating signatures of gene evolutionary history – paralogy/orthology, and codon biases – and create a large database of predicted gene functions usable for microbiologists.

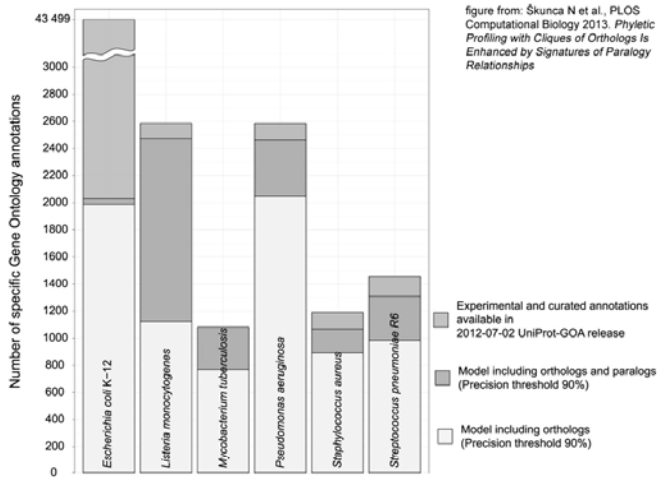
Methods: OMA ortholog groups were used in tandem with paralogy relationships inferred by the „witness of non-orthology" rule. The proteins' Gene Ontology categories were transferred across phyletic profiles using the CLUS hierarchical multilabel classifier based on decision trees. Significant codon biases were determined using a Random Forest test that compares against the composition of intergenic DNA. Codon biases in COG gene families were contrasted between microbes inhabiting different enviroments, while controlling for phylogenetic inertia.

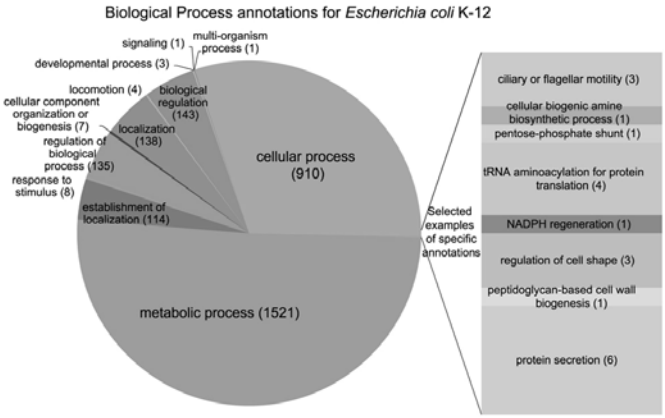
Results: Genomic co-occurence patterns of both orthologs and paralogs (the homologs separated by a speciation or duplication event, respectively) are informative and synergistic in a phyletic profiling setup [1]. We deposited the resulting ~400,000 gene function predictions for 998 prokaryotes (FDR<10%) in the GORBI web server (http://gorbi.irb.hr/) and experimentally validated the confidence levels reported therein using *E. coli* deletion mutants [1]. Finally, we introduce a novel 'genome context' method to systematically link codon adaptation in COG gene families to microbial phenotypes and environments (thus functionally characterizing the COGs) and experimentally verify the predicted *E. coli* genes relevant for oxidative, thermal or osmotic stresses.

Conclusion: Our work towards enhancing phyletic profiling and developing complementary genomic context approaches helps prioritize experimental investigation of microbial gene function, cutting time and cost needed for discovery.

[1] Škunca N et al. PLOS Comp Biol 2013







05

Ecological and evolutionary views on how bacterial diversity buffers pathogen invasion in soils

J. Falcão Salles[1], C. A. Mallon[1], F. Poly[2], X. Le Roux[2], J. D. van Elsas[1]
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[2]INRA, CNRS, Université Lyon 1 , Ecologie Microbienne (UMR 5557, USC 1193), Villuerbanne, France

The capacity of alien species to persist in new environments has been associated with the diversity of the indigenous communities. However, the mechanisms by which diversity buffers invasion remain underexplored. We hypothesized that a community with a greater diversity would extract more resources with greater speed than a less diverse community. This mechanism termed resource complementarity would in turn limit the invader's survival. To test that, we have manipulated the diversity of microbial communities by using assemblage and removal approaches, in soil microcosm experiments. After establishment, these soil communities were inoculated with the invading species *Escherichia coli* O157:H7, and we followed invaders fate for up to 75 days after inoculation. As expected, soil microbial diversity had a negative effect *E. coli* survival, and this effect became more evident towards the end of the experiments. *In vitro* competition experiments performed with communities of up to 20 species, either with or without the invader, indicated that species-rich communities had higher consumption rates than *E.coli*. Moreover, *E.coli*'s competitive potential, seen as the amount (R^2=0.46) and rate of carbon (C) use (R^2=0.33), decreased with increasing species richness. To verify the relevance of these findings in soil, we measured the potential amount and rates of C

utilization in the communities from the dilution to extinction experiments (10^{-1}, 10^{-3}, and 10^{-6}; 2 inocula: soils B and W). Overall, the amount of C utilized by the communities could explain a larger proportion of the variance in *E.coli* survival (62%) than utilization rates (35%). However for W communities both the amount and rates of C utilization equally explained *E.coli* survival (77-78%), whereas invaders survival on B communities could be explained by the amount of C sources (60%), but not by rate. Considering that the matrix used for the microcosm was sterile W soil, these results imply that the W community had an adaptive advantage over B community towards the invader. We conclude that from an ecological perspective, the amount of resources utilized by a community is the major mechanism by which diversity buffers invasion, whereas from an evolutionary perspective, both the amount and speed of resource utilization play equal roles.

06
Horizontal genetic transfer and the origin of bacterial species

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Closely related bacterial genomes usually differ in gene content, suggesting that nearly every strain in nature may be ecologically unique and that new, ecologically distinct species (ecotypes) are formed at an extremely high rate. Our laboratory microcosm experiments support this inference: bacteria in microcosms can diversify into new ecotypes as rapidly as adaptations accrue within an existing ecotype. We have further tested for rapid speciation by sequencing the genomes of extremely close relatives within a recognized taxon, and analyzing the genomes for evidence of ecological distinctness and a role for horizontal transfer in speciation. We compared the genomes of five isolates previously classified to *Bacillus subtilis* subsp. *spizizenii* and hypothesized through multilocus analysis to be members of the same ecotype, named Putative Ecotype 15 (PE15). Each of the strains was under a different regimen of positive selection on shared genes, suggesting that each strain is ecologically unique and represents a distinct ecological speciation event. The rate of speciation appears to be much faster than can be resolved with multilocus sequencing. Each PE15 strain contained unique genes, acquired through horizontal transfer and known to confer a function for bacteria. Remarkably, no unique gene conferred a metabolic system or subsystem function that was not already present in all the PE15 strains sampled. Thus, the origin of ecotypes within this clade shows no evidence of qualitative divergence in the set of resources utilized, much like speciation in animals. These results suggest more generally that while newly divergent ecotypes may differ in the functional genes they contain, through horizontal transfer, they may not gain new ecological functions that would allow the ecotypes to diverge irreversibly.

07
Bacterial species that are defined by genes but not by ecology

P. Young[1]
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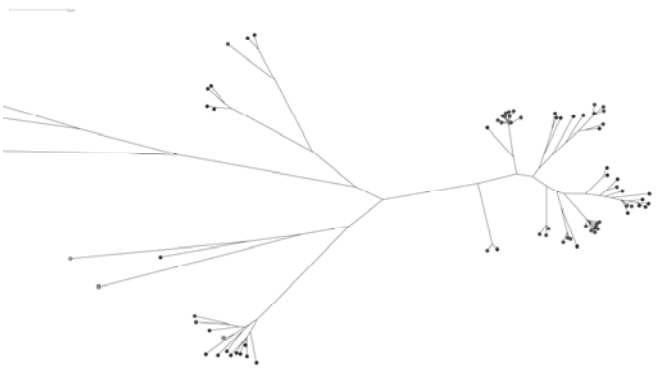
1. Introduction: Biological species may remain distinct because of genetic isolation or ecological adaptation, but these two aspects do not always coincide.

2. Objectives: To establish the nature of the species boundary and the role of HGT within a local bacterial population.

3. Materials and Methods: We describe a sympatric population of the bacterium *Rhizobium leguminosarum* by genomic sequencing of 72 isolates, half of which are symbiotic exclusively with clover, and half with vetch.

4. Results: Although all strains have 16S rRNA typical of *R. leguminosarum*, they are divided into five cryptic genospecies by the accepted criteria for genomic divergence. A large core of genes, on plasmids as well as the chomosome, supports this division; HGT of core genes has been largely within, rather than between genospecies. Nevertheless, variation in important ecological properties, including symbiotic host range and carbon-source utilization, cuts across these genospecies, so that no phenotype is diagnostic of genospecies. This phenotypic variation is conferred by islands of genes that move within and between genospecies.

5. Conclusion: The genospecies meet the Mayr criteria for Biological Species in respect of their core genes, but they do not correspond to coherent ecological groups. Some major suites of correlated adaptive traits, such as symbiotic host range, merit recognition as "biovars". This approach is widely applicable and provides a more natural description of some other problematic bacterial taxa. Bacterial species are traditionally defined by "polyphasic taxonomy", requiring both phylogenetic coherence and distinctive phenotypic traits, but this does not map well onto current understanding of the biology of bacteria. A consistent taxonomy of bacteria cannot combine both genomic and phenotypic criteria, and we argue that bacterial systematics should, in future, be based on core gene relationships without requiring that a species should necessarily be phenotypically homogeneous.



08
How have terrestrial thaumarchaea really evolved?

D. MacQueen[1], C. Tusche[2], A. Mc Hardy[2], C. Gubry-Rangin[1]
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[2]Max Planck Institute , Bioinformatic, Dusseldorf, Germany

Thaumarchaea are abundant in many ecosystems, including the soil, where they can play a major role in ammonia oxidation, a key step in the nitrogen cycle in which ammonia is converted to nitrite. Analysis of globally distributed database sequences of the *amoA* (ammonia monooxygenase) gene and high throughput sequencing of regional *amoA* sequences provided strong evidence that pH is the major driver of niche specialisation and community structure of these organisms (Gubry-Rangin *et al.*, 2011, PNAS). The four dominant phylogenetic clusters displayed strong soil pH preference, with 2 acidophilic (≤pH 5) and 2 neutrophilic (≥pH6) clusters.

While numerous studies have focused on the distribution and ecophysiology of thaumarchaea, their evolutionary history and ancestral characteristics have not been analysed. To address these issues in the context of the ecology of soil thaumarchaea, a study was performed with four major goals: (1) define the historical soil pH preference of the thaumarchaeal ancestor, (2) determine diversification rates (3) relate diversification rates to pH adaptation and (4) analyse the molecular signature related to pH adaptation.

Thaumarchaeal *amoA* sequences and contextual pH data were used to reconstruct the soil pH preferences of thaumarchaea along the course of their evolution (including the presumed thaumarchaeal ancestor) and to decipher distinct events of colonisation of acidic and alkaline environments. This analysis is congruent with detectable events of strong selective pressure, based on reconstructed ancestral sequences. These events are also related to important environmental changes, in terms of both ecosystem and soil pH. Past and current diversification rates of terrestrial thaumarchaea, and their relation to soil pH, were determined in different pH niches. Finally, detection of several codons under selection in specific dominant clusters and their representation on the multidimensional structure model of the protein demonstrates the influence of pH on the evolution of AMOA protein. Altogether, these results demonstrate how terrestrial thaumarchaea have evolved and offer new perspectives of research.

This study links the microbial ecology and the molecular evolution approaches in order to better understand the distribution of microbes in the environment.

09
Molecular authentication of the phage carrier state in *Salmonella* Typhimurium

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Background and Aims: Viruses of bacteria are the most abundant biological entity on earth, and have a tremendous impact on the ecology of their hosts. It has traditionally been recognized that upon phage infection the host cell is forced either to produce and release new virions during lytic development, or to replicate and segregate the phage chromosome together with its own genetic material during lysogenic development. These different developmental paths are orchestrated by a dedicated set of phage - host interactions that are able to sense and redirect host cell physiology. In addition to this classical bifurcation of phage development, however, many studies on phage biology in natural ecosystems also hypothesize the existence and significance of stable phage carrier (or pseudolysogenized) cells that are not engaged in either lytic or lysogenic proliferation. In this study we look for molecular evidence authenticating this alleged route in phage biology.

Results: Using *Salmonella* Typhimurium and phage P22 as a model system, live fluorescent imaging was performed to study phage infection dynamics throughout the population at the single cell level [1]. Aside cells undergoing typical lytic and lysogenic development, some cells were found to carry an unintegrated phage chromosome that segregated asymmetrically among carrier cell siblings. Importantly, this phage carrier state enabled the execution of a novel phage - host interaction, of which the molecular details are being dissected.

[1] Cenens *et al.* (2013). PLoS Genetics, 9:e1003269.

10
The evolution of social interactions in spatially organised bacterial communities

S. Mitri[1], K. Foster[1]
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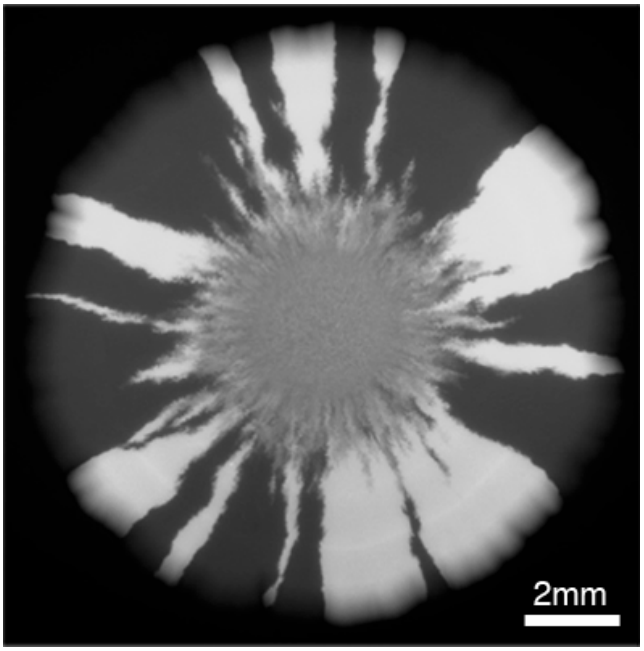
Microbes live in dense, surface-attached communities composed of different strains and species. Disentangling social interactions within these communities is central to understanding them. In particular, the spatial organisation of a community is expected to have strong effects on the evolution of positive or negative interactions between its members. Here, we ask what ecological factors determine the spatial organisation of a simple microbial community, and whether the resulting spatial patterns affect selection on cooperative phenotypes in the resident strains. Finally, we are interested whether increasing the number of strains influences these conclusions. We address these questions with competition experiments between two or more strains conducted using computational simulations and laboratory experiments with Pseudomonas aeruginosa strains (see Figure). We present data showing that the spatial organisation of a two-strain bacterial community depends on ecological factors, such as nutrient concentrations, which strongly affect selection for cooperative traits. Finally, we show that the presence of a third strain can influence both spatial structure and, in turn, selection for social phenotypes within the first two strains. These data represent the first steps toward developing a general theory of microbial social interactions in a spatial context. Ultimately, understanding how they evolve as a function of their ecology will allow us to manipulate microbial communities to our advantage.



11
Social evolution of microbes

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Since Darwin, evolutionary biologists have been fascinated by cooperative behavior. Honeybee workers labor their whole life without reproducing, birds make alarm calls, and humans often help one another. However, it is only recently that the full extent of microbial sociality has come to light. Microbes often live in large dense groups where one cell can strongly affect the survival and reproduction of others. But do microbes typically help or harm those around them and can we identify the factors that promote cooperation over competition? We study these questions using a diversity of systems, including computer simulations, pseudomonad bacteria and budding yeast. We find that single-genotype patches naturally emerge in microbial groups, which creates favorable conditions for cooperation within a particular genotype. Experimental evolution in bacteria shows that this process drives extremely strong natural selection for cooperative adaptations that can be understood at the molecular scale. Moreover, some microbes actively adjust both genotypic assortment and investment into social traits in a way that promotes cooperation within a genotype. However, our work on interactions between different microbial genotypes suggests that, here, the evolution of competitive phenotypes is more likely than cooperation. This leads us to a simple model - the genotypic view - that predicts microbes will evolve to help their own genotype but harm most other strains and species that they meet.

12
Less is more – bacterial gene loss results in division of labour and the formation of intercellular networks

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[1]Max Planck Institute for Chemical Ecology, Experimental Ecology and Evolution, Jena, Germany
[2]Friedrich Schiller University Jena, Bioinformatics, Jena, Germany
[3]Friedrich Schiller University Jena, Theoretical Systems Biology, Jena, Germany
[4]Max Planck Institute for Chemical Ecology, Biochemistry, Jena, Germany
[5]European Bioinformatics Institute, Cheminformatics and Metabolism, Cambridge, Great Britain
[6]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

Cross-feeding interactions, in which bacterial cells exchange metabolites to the benefit of the interacting partners, are very common in the microbial world and have been shown to readily evolve under laboratory conditions. This type of ecological interactions, however, is inherently difficult to study within natural microbial communities, because of the complexity to identify the chemical nature of the interaction as well as difficulties to determine the underlying genetic basis. To solve this problem, we engineered synthetic cross-feeding interactions between two genotypes of *Escherichia coli*. The design of these interactions was guided by prior knowledge on essential features of the focal interactions as well as computational analyses to identify genetic targets. By simply deleting two metabolic genes, we generated a range of genotypes that reciprocally exchanged essential amino acids when cocultured. Surprisingly, in a vast majority of cases, cocultures of two of these cross-feeding strains showed an increased Darwinian fitness relative to unmutated wild type (WT) cells - even in direct competition. This unexpected growth advantage was due to a division of metabolic labour among cooperating cells: the fitness cost of overproducing certain amino acids was less than the energetic gain of not having to produce others when they were provided by their partner. Interestingly, in spatially structured environments (i.e. agar plates), in which amino acids are distributed more locally, cross-feeding consortia could persist and even outcompete non-cooperating types (i.e. WT or auxtrophs), while in a spatially unstructured environment (i.e. liquid culture), cross-feeding was the least fit strategy. This result emerged both in theoretical, individual-based models as well as in coculture experiments. Our finding provides an adaptive explanation for the ease, with which bacteria enter into metabolic mutualisms with other micro- or macroorganisms and suggest bacteria most likely function as a network of interacting cells, rather than as physiologically autonomous units.

13
Social networking pays off

P. Stefanic[1], A. Oslizlo[1], B. Kraigher[1], I. Dogša[1], I. Mandic-Mulec[1]
[1]University of Ljubljana, Biotechnical faculty, Ljubljana, Slovenia

Bacillus subtilis communicates through a highly polymorphic *comQXPA* quorum sensing (QS) system, which regulates genes expressed during the early stationary phase. The *comQXP'* polymorphism leads to four communication groups (pherotypes). Strains of the same pherotype communicate efficiently while those of different pherotypes do not, even if they are highly related at the phylogenetic level and isolated from the soil microscale. The Ecotype Simulation (ES) algorithm based on *gyrA*, *rpoB*, *dnaJ* genes indicated 3 putative ecotypes with significant but not exclusive overlap between ecotypes and pherotypes of microscale strains (Stefanic et al, 2012). Consistently with ecotype demarcation based on phylogenetic markers we now show that ecotypes and pherotypes at least indirectly, influence appearance of self recognition patterns and the formation of separation zones

between two swarm colonies on semi solid media. However, despite the dramatic polymorphism of QS loci, we have not found any *B.subtilis* strain that would possess a dysfunctional ComQXPA QS system until now. This suggests a strong selective pressure for functional social interactions and high fitness cost of signalling mutants. To test this hypothesis we measured fitness traits of QS wild type and QS signal deficient mutants in pure and co-cultures. Our studies revealed that signal deficient strains show a significant decrease in fitness as compared to the wild type, but most notably when in a social context with the wild type. Also, if purified signalling peptide ComX was added to the signal deficient mutant its fitness decreased and its secondary metabolism was over-induced affecting the well being of the cells participating in the quorum sensing network. We here propose that the fitness loss of *comQ* and *comX* mutants, by being dependent on social interactions and coupled to metabolic prudence, may play a selective force behind the striking polymorphism of this QS system.

Stefanic, P, Decorosi F, Viti C., Petito J.,Cohan F., I. Mandic Mulec (2012) The quorum sensing diversity within and between ecotypes of Bacillus subtilis. *Environ. microbiol.* vol. 14: 1378-1389.

14

Bacterial sense of small – the role of volatiles as infochemicals in bacterial inter-specific interactions

P. Garbeva[1], W. de Boer[1]
[1]NIOO-KNAW, Microbial Ecology, Wageningen, Netherlands

The importance of bacterial interactions in microbial ecology is increasingly recognized. However, little attention is paid to the role of bacterial volatiles in microbial interactions. Gas-filled pores are an important constituent of most soils and therefore, volatiles may have a prominent role in the interactions between relatively distantly distributed soil microorganisms.

In order to obtain insight in the importance of volatiles in soil microbial interactions we performed sand microcosm experiments in Petri dishes plates, which were designed as such that growth of different microorganisms occurred in physically separated areas within a common atmosphere. We determined the population dynamics, behavior and gene expression of the model soil isolate *Pseudomonas fluorescens* Pf0-1 exposed to volatiles produced by different mono- and mixed cultures of several soil bacteria. The produced volatiles were collected in a steel trap (filled with Tenax TA and Carbopack B) than desorbed from the trap using automated thermodesorption unit and analyzed with the GC-MS.

The composition of the volatiles produced by different monocultures was different. Microarray-based analyses indicated strongly different responses in the gene expression of *P. fluorescens* Pf0-1 when exposed to the volatiles of different soil bacterial species. Differentially expressed genes were mainly involved in amino acid transport and metabolism, signal transduction mechanisms, inorganic ion transport and metabolism, cell motility and secretion and energy production and conversion.

The obtained results supported the important role of volatiles as infochemicals in soil bacterial interactions.

15

Enhanced biofilm formation of bacterial consortia from various natural habitats suggest ubiquity of synergism in multispecies biofilm

M. Burmølle[1], J. Stenløkke Madsen[1], D. Ren[1], H. Lyng Røder[1]
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Multispecies biofilms are predominant in almost all natural environments, setting the scene for various competitive and cooperative interactions that affect overall functionality and fitness of the individual strains and the community.

We have developed a model defining synergism, antagonism and neutrality within multispecies biofilms, based on the proportion of the species that are present and their ability to form monospecies biofilm. The model was validated experimentally in control systems of isogenic strains differing only in their ability of biofilm formation as single species. We applied this model to characterize the interactions in multispecies biofilm formation of bacterial consortia isolated from a variety of natural habitats. In brief, strains were co-cultured in microtiter plate wells and biofilm was assessed by a modified version of the Calgary method based on crystal violet retention and quantification. Biofilm formation of single strains and all possible combinations of 2 -7 strains were compared. Synergism in multispecies biofilm formation was observed within all strain collections, ranging from 20 - 80 % of all combinations tested.

In order to further characterize the observed synergistic interaction, we explored a four species community composed of the soil isolates *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans* and *Paenibacillus amylolyticus*. When co-cultured, these strains produced almost five-fold more biofilm biomass compared to single species biofilms. It was observed that each of the four strains was indispensable for the synergy to occur. However, q-PCR proved that the four strains were represented in the multispecies consortium in very different proportions: 1000:500:5:4, suggesting significant roles for both low- and high-abundance strains.

In conclusion, we present results indicating ubiquity of synergism in multispecies biofilm formation in complex bacterial communities. Furthermore, we have developed and applied a protocol for assessing the strain composition in multispecies biofilms, useful for characterizing the underlying interspecific interaction as being of competitive or cooperative nature.

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Exploiting the rhizosphere microbiome to improve crop production

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Microbial communities that are associated with plant roots are highly diverse and harbor incredible numbers of bacterial and archaeal species. Several functions sustained by this so-called rhizosphere microbiome are drivers of plant health and include the suppression of infectious diseases. The latter function is prominent in disease suppressive soils. From such soils micro-

organisms have been selected that can effectively control soil borne diseases. The mechanisms implicated in disease suppression by these biological control agents include competition for nutrients and space, antibiosis, and induced systemic resistance (ISR). For many biological control agents, and especially fluorescent *Pseudomonas* spp., ISR has been recognized as a major mechanism of disease suppression. ISR eliciting traits of fluorescent *Pseudomonas* spp. have been identified, they are diverse and in many cases there is redundancy of ISR elicitors. Here we will discuss implications of induced resistance on the ecology of ISR eliciting biocontrol agents and on the recruitment and functioning of the rhizosphere microbiome.

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Sphagnum-associated microbiome – Treasure chest or Pandora's box?

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Bryophytes of the genus *Sphagnum* play a crucial role in biosphere carbon sequestration and harbor highly diverse, mostly undescribed microbiome. Applying a combination of high throughput molecular methods and cultivation approaches, we studied the bacterial biodiversity of cosmopolitan *Sphagnum* species (*S. magellanicum*, *S. fallax* and *S. angustifolium)* from different bog ecosystems in Austria and Russia with a special focus on their ecology and function.

Dense colonization patterns of Sphagna were detected by fluorescent *in situ* hybridization (FISH) coupled with confocal laser scanning microscopy (CLSM), especially in hyaline cells of leaves and cortical cells of stems. Microbiome composition was highly specific for each *Sphagnum* species at the local as well as multi-geographical scales. The pH and nutrient richness were defined as the main environmental parameters driving the microbial diversity. Moreover, transfer of a core microbiome from one generation to the other within the sporangium capsules was demonstrated by analyzing the FISH-CLSM and deep-sequencing datasets. The ecosystem function of the *Sphagnum* microbiome was explored by cultivation survey, deep-sequencing and quantification of functional key genes as well as metagenome analysis. We identified a high diversity of bacteria important for nutrient supply to the host plants, defense against pathogens but also for reducing the greenhouse emissions which are relevant for the world climate. For the first time, we demonstrated plant-specificity of the functional microbial patterns determined by their function within the ecosystem.

Taking together, bryophytes of the genus *Sphagnum* harbor highly diverse and specific microbial communities that are beneficial for the plant growth and health, and positively contribute to the ecosystem sustainability. Therefore, *Sphagnum*-associated microbiomes represent a treasure chest for ecological studies but also for diverse biotechnological applications.

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Stability of multispecies bacterial communities – signaling networks may stabilize microbiomes.

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Introduction: Multispecies bacterial communities consist of cells and species that compete for environmental resources, however the community itself can be remarkably stable over time and resilient with respect to environmental challenges. In certain diseases, small cohorts of bacterial species that understand each other's signals appear to mediate disease For instance, in the olive-knot disease of the olive tree, the causative agent *Pseudomonas savastanoi pv. savastanoi* (PSV) is frequently associated with non-pathogenic *Pantoea agglomerans* and *Erwinia toletana* (ET) [1].

Objectives: We aim to use in silico models for understanding some general principles underlying the stability of multispecies communities, with particular regard to those properties that are hard or impossible to test by experiment.

Results: *C*ommon signals released into the environment appear to help various bacterial species cluster at common locations and that sharing of public goods stabilizes this coexistence. In contrast, unilateral eavesdropping on signals produced by a potentially invading species may protect a community by keeping invaders away from limited resources [2]. Shared bacterial signals, such as those found in quorum sensing systems, may thus play a key role in fine tuning competition and cooperation within multi-bacterial communities [3].

Conclusions: We suggest that in addition to metabolic complementarity, signaling dynamics may be important in further understanding complex bacterial communities such as the human, animal as well as plant microbiomes.

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Ecology of bacteria associated with plant litter – decomposers, cheaters and mycophages

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Fungi are often regarded as the drivers of litter transformation in terrestrial ecosystems due to their ability to produce extracellular enzymes. Indeed, fungal biomass in litter is high and fungal

communities on decomposing litter are diverse and dynamic. Far less is known about the ecology of litter-associated bacteria, although bacterial abundance on litter is also high. Here we present a summary of data on the composition of bacterial communities in litters ranging from grasslands along the successional stages of decomposition. These are supplemented with targeted experiments aiming at the characterization of active bacteria (based on RNA rather than DNA quantification) and their role in litter decomposition explored by means of Stable Isotope Probing. The results show that bacterial/fungal biomass ratio tends to increase with litter age in all studied decomposition series. Bacterial communities are more even than fungal communities and the successional changes with litter age are in general less pronounced. Also, the most important bacterial genera associated with litter occur on different litter types. While fungal communities are litter type-specific, bacterial communities tend to be shaped rather by general litter chemistry (e.g., pH, N-content, lignin content etc.) Stable isotope labelling shows that many bacterial taxa are efficient decomposers of plant organic matter mediating a significant share of decomposition. Interestingly, communities of bacterial decomposers of cellulose and hemicellulose in forest litter are different. In addition to decomposers, bacterial communities on litter also harbor taxa that use the products of fungal decomposition (i.e., cheaters), and mycophagous bacteria frequently occur making use of the high amount of fungal biomass in the decomposing litter. Our results indicate that bacterial communities associated with various litters share several properties and are functionally important in organic matter transformation.

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Are microbial communities the sum of their parts? – dismantling and reassembling the Drosophila gut microbiota

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It is well-established that the gut microbiota influences multiple traits of the animal host, including nutrition, immunity and behavior. The next challenge is to identify the microbial taxa and activities that mediate these effects. Our strategy is founded on two alternative hypotheses and an experimental system in which the association can be disassembled and reassembled with ease.

We define microbiota function as the difference in a host trait between untreated animals containing the microbiota and germ-free animals. Our hypotheses are that microbiota function (1) is highly interactive, i.e. microbiota impact on host traits requires multiple, interacting microbial taxa, or (2) is mediated by single taxa, such that different functions can be coupled precisely to specific microbial taxa. These alternatives may be extremes of a continuum, and different microbiota functions may vary in the identity and number of contributing taxa. Understanding patterns in the relationship between microbial taxa and function is crucial to explain and predict microbial impacts on host fitness.

Our experimental system is Drosophila, which has a gut microbiota of low taxonomic diversity, dominated by a handful of bacterial species that are readily cultured. We have developed procedures for generating germ-free (axenic) D. melanogaster, and for re-associating these axenic hosts with cultured bacteria to generate gnotobiotic flies. For this study, we characterized the impact of eliminating the microbiota on a range of host traits. Then, by generating gnotobiotic flies with five, single-species microbiota, we tested the hypothesis that some functions of the microbiota can be mediated by a single taxon.

Our comparison of germ-free and conventional flies identified multiple microbiota functions: promotion of developmental rate and survival to adulthood on low-nutrient diets, regulation of body glucose and lipid levels, and up-regulation of immune genes, especially anti-microbial peptides. The pattern of traits in gnotobiotic flies colonized by each of the five dominant bacteria derived from the gut microbiota of untreated hosts demonstrated that some functions can be attributed to specific individual taxa. For example, hosts from all gnotobiotic treatments with Acetobacter species developed at the same rate as conventional hosts while some single-species treatments with Lactobacilli delay development relative to conventional rearing. Other traits have an interactive basis, being dependent on multiple bacteria.

Our analysis provides an experimental framework for testing how changes in the microbiota composition, induced by diet, disease, age etc., can influence animal health and fitness. Furthermore, in combination with genome sequences from all of our gut isolates, these data generate specific, testable hypotheses on the mechanisms underlying microbe-dependent impacts on host traits.

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A spatial examination of the bacterial community diversity and lignocellulose composition of wood present in the gastrointestinal tract of *Panaque nigrolineatus*, a wood-eating catfish

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Xylophagy -ingestion of wood for nutritional benefit generally requires the activity of specialized enteric microbial communities capable of lignocellulose degradation and nitrogen fixation. The refractory and nitrogen-limiting nature of lignocellulose makes xylophagy an exceedingly rare trophic strategy among animals. The armored catfish, *Panaque nigrolineatus*, has specialized physiological adaptations to enable high levels of wood to be ingested in its diet. However, it is unclear what nutritional benefit is derived or the nature of any symbiosis that facilitates it. In the present study, we examined microbial communities associated with the foregut, midgut, hindgut, and auxiliary lobe of *P. nigrolineatus* using a metagenomic approach utilizing 454 pyrosequencing. Microbial community diversity altered in the different regions of the GI tract indicating different functional roles of key bacterial populations. To complement this genetic approach, scanning electron microscopy of the different regions of the gastrointestinal tract was performed to examine the microbial community present. Lignocellulose degradation within the gastrointestinal tract was supported by scanning electron microscopy observations, indicating structural alterations in the wood. Furthermore, the wood particles in the hindgut appeared to harbour assemblies suggestive of microbial cells. Additionally, wood collected from different regions of the GI tract was analyzed using Fourier-Transformed Infrared (FTIR) spectroscopy and X-ray powder diffraction. These combined techniques revealed changes to the wood associated with gut transit. These changes are consistent with the presence of a wood-

digesting activity within the GI tract within the *P. nigrolineatus* gastrointestinal tract.

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Insights into the bovine rumen plasmidome

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Plasmids are self-replicating genetic elements capable of mobilization between different hosts. They often serve as mediators of lateral gene transfer, a process considered to be a strong and sculpting evolutionary force in microbial environments. Our aim was to characterize the overall plasmid population in the environment of the bovine rumen, which houses a complex and dense microbiota that holds enormous significance for humans. We developed a procedure for the isolation of total rumen plasmid DNA, termed rumen plasmidome, and subjected it to deep sequencing using the Illumina paired end protocol and analysis using public and custom-made bioinformatics tools. A large number of plasmidome contigs aligned with plasmids of rumen bacteria isolated from different locations and at various time points, suggesting that not only the bacterial taxa, but also their plasmids, are defined by the ecological niche. The bacterial phylum distribution of the plasmidome was different from that of the rumen bacterial taxa. Nevertheless, both shared a dominance of the phyla Firmicutes, Bacteroidetes and Proteobacteria. Evidently, the rumen plasmidome is of a highly mosaic nature which can cross phyla. Interestingly, when we compared the functional profile of the rumen plasmidome to two plasmid databases and two recently published rumen metagenomes, it became apparent that the rumen plasmidome codes for functions which are enriched in the rumen ecological niche and could confer advantage to their hosts, suggesting that the functional profiles of mobile genetic elements are associated with their environment, as has been previously implied for viruses.

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Functional specificities of microorganisms on two different human skin body sites

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Human skin microbial communities that might impact skin appearance and health are now being investigated by metagenomic approaches. Direct microbial DNA extraction and sequencing of *rrs* ("16S") gene PCR products has characterized an extended part of the diversity of microorganisms colonizing different sites on the human skin. Apparent taxonomical differences would suggest that the substrates available in the various skin environments provide specific adaptive environmental conditions for microorganisms related to their functional specificities. In this study, we built metagenomic datasets through DNA extraction and high throughput (NGS) sequencing from long term extraction of forehead and foot skin microbial communities without any prior PCR amplification. Metagenomic sequences were anotated for their association with different functional subsystems as well as specific protein families associated with the degradation of the chemical consituents of human skin. Functional differences were detected between sebaceous and wet environments yielding coherent explanations concerning the ecology of these microorganisms and possibly providing observations that could improve our understanding of their relationships with their host.

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The big picture – lessons from a rapidly expanding genomic tree of life

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Most microorganisms cannot be grown in pure culture (or at least not easily). This has been apparent for decades by comparing the number of cells seen under a microscope to the fraction of those cells that will grow into colony forming units (typically <1%). The advent of culture-independent rRNA-based molecular surveys pioneered by Norman Pace put our degree of ignorance of the microbial world into perspective: dozens of major microbial lineages have emerged over the last 20 years that lack even a single cultured representative. New approaches, such as deep metagenomics and single cell genomics, are now transforming the rRNA-based phylogenetic outlines of the tree of life into a fully fledged genome-based view of the tree. I will present a snapshot overview of the genome tree of the bacterial and archaeal domains and examples of functional insights in the context of a more complete view of microbial evolution.

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Responses of soil bacterial and fungal communities to extreme soil drought and rewetting

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Question: The patterns of resource allocation and activity of the soil microbial community over the dry summer in Mediterranean grasslands are still largely unknown. The microbial response to summer desiccation reflects adaptation strategies, setting the stage for a large rainfall-induced soil CO_2 pulse upon rewetting, an important component of the ecosystem carbon budget.

Methods: In three California annual grasslands, the present (DNA-based) and potentially active (RNA-based) soil bacterial and fungal communities were tracked over a summer season and in response to controlled rewetting of intact soil cores. Phylogenetic marker genes for bacterial (16S) and fungal (28S) RNA and DNA were sequenced and the abundance of these genes and transcripts were measured.

Results: While bacterial community composition differed between sites, all sites shared a similar response pattern of the present and potentially active bacterial community to dry-down and wet-up. In contrast, the fungal community was not detectably different between sites, and largely unaffected by drought, showing a marked resistance to dessication. The potentially active bacterial community changed significantly as summer drought

progressed, then returned to pre-drought composition within several hours of rewetting, displaying spectacular resilience. Upon rewetting, transcript copies of bacterial *rpoB* genes increased consistently, reflecting rapid activity resumption. Changes in relative abundance of the most dominant potentially active bacterial taxa reflected a differential response of phyla, which was consistent across sites and conserved at high taxonomic level.

Conclusions: These contrasting drought-related bacterial life-strategies suggest that predicted changes in precipitation patterns may affect soil nutrient and carbon cycling by impacting activity patterns of microbial communities.

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Aggregate-scale spatial distribution of extracellular enzymes and microbial diversity in soil

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Introduction: Soil organic matter (SOM) does not breakdown spontaneously by itself. Its breakdown is catalysed by extracellular enzymes that are produced by microorganisms. Extracellular enzyme activities (EEA) are responsible for the rate-limiting steps in the dynamics of SOM. The spatial distribution of EEA at the microbial micro-habitat scale is likely to influence the rate at which enzymes and substrate encounter each other and so influence the rate of SOM breakdown. It is likely therefore that the distribution of EEA constitutes a mechanistic parameter of biogeochemical soil functioning.

Objective: The objective of this work was to characterize the spatial distributions of a range of EEA at the micrometer to millimeter scale and to determine how these were affected by soil management in relation to the effects of soil texture. This was achieved by comparing organically, without fertilizer or pesticide use, and conventionally managed loamy soil and a conventionally managed clay soil.

Material and Methods: The presence of 7 EEA (oxydases or hydrolases) involved in the C, N or P cycles in soil aggregates was measured by fluorescence in microplates. The spatial distribution was measured in 48 aggregates of 0.25, 0.5, 1, 2, 3-mm diameter. The type of distribution (spatially aggregated or dispersed) was quantified using the the frequency of EEA presence in each aggregate size class.

Results: Glucosidase, xylosidase, cellobiohydrolase, chitinase were more spatially aggregated under conventional than organic management. EEA were more spatially dispersed in the clay than in the loamy soil, regardless of management. Alkaline Phosphatase was the most dispersed in all the soils. The enzyme presence pattern and the bacterial diversity in aggregates showed no relationship, suggesting functional redundancy in microbial communities at these scales. The influence of soil management practices and of soil type on the spatial distribution of EEA was of the same order of magnitude.

Conclusion: The spatial distribution characteristics indicate a deterministic behaviour of the distribution of enzymes which is modified by soil management practices and indicate that certain types of SOM transformations are more susceptible to soil perturbation than others. These are useful information for modelling and soil fertility index.

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Bacterial laccases in genomes and metagenomes: from genes to functional enzymes

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Laccases are versatile enzymes that oxidize a variety of phenolic compounds. They have been found in all domains of life but have been most extensively studied in fungi where they play a vital role in the degradation of lignocellulose but also in fungal morphology and lifestyle. Bacterial laccase-like enzymes are much more diverse than the fungal enzymes, both in terms of structure and activity, while their function is largely unknown. Our aim was to produce novel bacterial laccases both from (i) existing sources (cultivated bacterial strains) and (ii) bulk environmental diversity.

Firstly, bioinformatics was used find over 1200 putative laccase genes in the sequenced genomes. Selected genes from bacteria with the desired lifestyle were expressed in *E. coli*, the recombinant proteins were purified and characterized to determine the optimal conditions for their activity. Secondly, a metagenomic library from high organic and low pH bog soil was prepared and screened using a PCR-based molecular approach. Full length genes obtained from individual clones were expressed and the enzymes characterized as described above.

Three novel bacterial laccases have been isolated so far. One is from an autotrophic alkaliphilic bacterium *Thioalkalivibrio sp*. It is capable of oxidizing a variety of substrates at alkaline pH. The second is from *Geobacter metallireducens*. This strain produces a thermostable laccase, even though it thrives in anaerobic or microaerophilic conditions. The third laccase proves that metagenomics can produce the full-length genes that were previously identified only in fragments of laccase targeted gene libraries prepared from bog soil.

The present study adds to our understanding of the diversity of bacterial laccases by exploring their activity rather than just sequences. It also unravels cues regarding the possible function of these enzymes *in vivo*, and suggest that they may be potentially applicable in biotechnology.

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Genome discovery from managed microbial communities

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Investigating environmental microbial communities based on metagenomic approaches would be improved by reducing the bottleneck of genome reconstruction from highly biodiverse ecosystems. Soil is a typical example where individual microorganisms are mostly uncultured and metagenomic datasets largely unassembled due to the even representation of the microbial community members. Here, the microbial community composition of a natural grassland soil was altered by applying ten different selective pressures to enrich different sub-populations and to extract, sequence, and assemble genetic structures from the complex metagenome. All tested conditions produced distinct metagenomic datasets. Seventeen genomes were successfully reconstructed using only eight gigabases of sequences and they represent about 0.05% of the natural soil metagenome recovered so far. The genomes belong to *Leifsonia*, *Rhodanobacter*, *Sporolactobacillus*, *Ktedonobacter*, *Streptomyces* and *Burkholderia* genera. Their relative proportions ranged from less than 10^{-4} % to 2.10^{-2} % of the natural soil microbial community and from 2% to 58% of their specific microcosm microbial community after four months of *in situ* enrichment. Several genes directly related to selective pressures were found in these genomes (mostly in large plasmids). Functions of potential industrial interest (*e.g.*, novel polyketide synthase modules in *Streptomyces*) were also discovered. In addition, possible massive phage infections were detected. This "divide and conquer" strategy using artificially induced stress to reduce biodiversity could be applied more extensively to soil as well as to other highly biodiverse ecosystems, and therefore, generate both rare and predominant genomes.

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Nutrient turnover and food web structures in soils with different history of rice cultivation in China

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Microbes are drivers for important ecosystem functions from soils, including crop quality and yield. Vice versa agricultural management highly influences microbial community structure and function soil. Furthermore site specific parameters like soil type or climatic conditions shape the performance of all biota in soil. Therefore there is a need to understand how microbial community structure and consequently the genetic potential of a soil changes over longer time periods in response to the agricultural management.

In the present study we addressed this question by investigating how one type of land management changes microbial community structure over a period of 2000 years based on a study performed along a chronosequence of soils that have been used as paddy soils for rice cultivation for different time periods. As in many areas of China, tidal wetlands have been continuously converted over time into agricultural land for rice cultivation, we were able to identify sites that have been under paddy rice cultivation for 50, 100, 300, 500, 1000 and 2000 years.

We used a polyphasic approach to investigate microbial community structure and function as influenced by the time of management including targeted approaches using specific primers to characterize abundance and diversity of well characterized processes like nitrification, N-fixation or denitrification as well as non-targeted methods based on direct sequencing of extracted DNA using 454 sequencing to reconstruct metabolic networks in the different soil samples. Samples were analyzed from field sites as well as from green house experiments under controlled conditions.

Overall we could clearly identify three phases of paddy soil evolution: The conversion of the tidal wetlands into paddy soils was followed by a significant increase in abundance and diversity of mainly nitrogen fixing microbes and microbes involved in nitrate ammonification (50 years paddy rice cultivation), the second phase was characterized as a transition phase were significant changes were observed compared to the earlier time points and the tidal wetlands in the community structure of facultative anaerobe microbes (100 - 500 years paddy cultivation). The last phase (

To our surprise with ongoing rice cultivation a significant increase of genes involved in plant pathogensis like type three secretions systems and other virulence genes could be detected.

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Is richness important for the functional performance of microbial communities?

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Question: Microbial communities provide important services to ecosystems and human society. These communities often contain many thousands of different microbial taxa, but it remains unclear whether taxonomic richness is an important determinant of their functional performance. Theory predicts that taxonomic richness should positively associate with the rates of microbial community functions. However, this prediction implicitly assumes that taxonomic richness also associates with functional richness. The goal of this research was to experimentally test this prediction and assumption.

Methods: We obtained ten different microbial communities from ten independent wastewater treatment plant (WWTP) microbial communities. We measured taxonomic richness for each community by sequencing and classifying bacterial 16S rRNAs. In parallel, we measured functional richness for each community by metatranscriptome sequencing and classifying mRNAs. Finally, we measured the rates of nine different pollutant biotransfomations for each community. We then tested for statistical associations between taxonomic richness, functional richness, and the rates of the different pollutant biotransformations.

Results: We found that both taxonomc and functional richness associate with the rates of pollutant biotransformations, but the strength of the assocations depend on the specific type of biotransformation. Taxonomic and functional richness are more likely to assoicate with oxidation biotransformations than with

hydrolysis biotransformations. We postulate that this bipartite behaviour is caused by how the different types of functions are distributed across different microbial taxa. We also found that taxonomic and functional richness associate with each other, thus confirming a key implied assumption of the theoretical predictions.

Conclusions: Our results demonstrate that both taxonomic and functional richness can predict the rates of specific microbial community functions. In addition, our results demonstrate that taxonomic and functional richness associate with each other, thus supporting the hypothesis that taxonomic and functional richness are important performance determinants of microbial communities.

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Reconstructing bacterial and viral genomes using metagenomics

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Metagenomic studies have shown that sequencing of a cellular metagenomic fosmid library results in retrieval of not only those prokaryotic populations that are underrepresented in the microbial community, but also a significant number of genomic fragments from phages. We have sequenced ~6000 metagenomic fosmids from the Mediterranean Deep Chlorophyll Maximum (MedDCM) using Illumina in batches of 250 fosmids to ensure efficient assembly. Analysis of these fosmids revealed a deep-branching lineage of marine Actinobacteria with very low GC content (33%) and the smallest free living cells described yet (cell volume ca. 0.013 μm^3), even smaller than the cosmopolitan marine photoheterotrophic, *Candidatus* Pelagibacter. A virtual genome reconstruction also indicated a very small genome size below 1Mb. A new kind of rhodopsin gene was detected indicating a photo-heterotrophic lifestyle. They are estimated to be ~4% of the total numbers of cells at the Mediterranean DCM and similar numbers were estimated in all tropical and temperate photic zone metagenomes available. Fosmids also provide an important alternative route to phages as phage biomass is difficult to retrieve from oligotrophic marine waters in amounts required to construct metaviriomes. Using nearly 1000 phage genome fragments assembled from this study, we have extracted complete phage genomes including some new groups of cyanophages, new groups infecting Pelagibacter and other uncultivated marine microbes. These genomes represent the first genomes of marine phages obtained by culture-independent methods and show that fosmid cloning from cellular metagenomes is a credible alternative to constructing metaviriomes, allowing capture and assembly of novel, complete phage genomes.

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Approaching an ecosystem from the top down and bottom up: using multi omic approaches to investigate the gut.

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In many systems the inability to grow the majority of the resident microbes or the logistical impracticalities of growing them, means we are increasingly using "omic" platforms to interrogate these systems and determine their diversity, dynamics and functions. With the considerable interest in the human microbiome increasing over the past decade, this has never been more true. The use of omic methods such as meta-taxonomics (16S rRNA gene inventories) and metagenomics has provided valuable insights into these systems, but in this talk I will explore how combining two omic approaches, at each end of the information spectrum, gives us better insights into ecosystem function.

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Gastrointestinal behavior of *Butyricicoccus pullicaecorum*, a novel butyrate producing isolate with probiotic potential in inflammatory bowel diseases

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Inflammatory bowel diseases (IBD) are characterized by a chronic inflammation of the gastrointestinal tract (GIT). IBD patients have both on phylogenetic as on functional level a dysbiosis. Therefore, manipulation of this unbalanced microbiota is an attractive therapeutic strategy.

The level of butyrate, a microbial metabolite with well-described beneficial effects in the gut, is decreased in IBD. Administration of butyrate-producing bacteria that are able to colonize the gut is therefore a promising treatment strategy.

Recently, a novel butyrate-producing bacterium, *Butyricicoccus pullicaecorum*, was isolated and characterized as a very potent butyrate-producing microorganism. The aim of this study was to evaluate this novel potential probiotic strain for its survival during passage of the upper GIT and its effect on the butyrate levels after reaching the colon. These properties are essential to make the *B. pullicaecorum* a suitable probiotic strain.

The survival experiments were executed in static batch incubations under stomach and small intestine conditions. The colon incubations were performed in a dynamic *in vitro* model for the human intestinal microbial ecosystem, the M-SHIME®. The model consisted of 4 vessels in parallel with the gut microbiota of 4 different donors. After stabilization, each colon vessel was treated with *B. pullicaecorum*. For the next 10 days the butyrate levels and composition of the microbial communities was followed and compared with the control (M-SHIME® with the microbiota of the same 4 donors without treatment).

The batch experiments showed that *B. pullicaecorum* is able to survive the conditions of the stomach and small intestine (9.15±0.6

log intact cells/mL). Plate counts and flow cytometry in parallel showed a viable but non culturable state during acid conditions of the stomach. Treatment of 4 M-SHIME® vessels with *B. pullicaecorum* resulted in higher butyrate levels in 2 of the 4 donor microbial communities.

We can conclude that *B. pullicaecorum* is still a promising candidate as a new probiotic strain in IBD. A high amount of viable *B. pullicaecorum* cells can reach the lower GIT after upper GIT conditions. Treatment of a M-SHIME® with *B. pullicaecorum* resulted in higher butyrate levels, demonstrating the potential of *B. pullicaecorum* to colonize a mixed intestinal microbiota.

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Bacterial chitinase with phytopathogen control capacity from suppressive soil isolated by functional metagenomics

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Introduction: Disease of plants caused by fungal pathogens contributes to extensive loss globally of crops important for food and energy production. Monoculture practices may further increase opportunities for the invasion of phytopathogens. As a consequence, the application of synthetic fungicides, many of which are toxic, is extensive and evidently has costs to public and ecosystem health. As a solution, microbial communities of soil suppressive to fungal crop disease may provide a source for the identification of novel enzymes functioning as bioshields against the plant pathogens.

Objectives: We targeted chitin degrading enzymes of the uncultured bacterial community through a genetic and functional metagenomics approach, developed under collaboration within the EU funded Metaexplore consortium.

Methods: A fosmid library of a suppressive field soil metagenome from Sweden was analyzed for chitinase genes using both activity based expression screening and genetic screening.

Results: A novel bacterial chitinase, Chi18H8, with antifungal activity against several important crop pathogens was identified. Chi18H8 was expressed in *E. coli* and other alternative hosts and purified by affinity chromatography. Enzyme characterization shows that Chi18H8 has a prevalent chitobiosidase activity (maximum activity at 35°C and at pH below 6) suggesting its role as exochitinase on native chitin.

Conclusion: To our knowledge, Chi18H8 is the first chitinase obtained in pure form from a metagenome library and that has the potential to be used as a candidate agent for controlling fungal crop diseases. Chi18H8 could also be a useful biocatalysist in various other industrial and medical purposes where there is a demand of an exochitinase.

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Mining bacterial inter-specific interactions for discovering novel antimicrobial compounds

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Recent genome sequencing data have shown that soil bacteria possess an extensive repertoire of genes encoding for secondary metabolites. However many of these compounds are not isolated so far. Most screening methods for the discovery of novel antimicrobials often target only monocultures of well examined microbial genera e.g. *Streptomyces* sp. already known as producers of antimicrobials without considering competitive inter-specific competitive interactions at which microorganisms naturally produce antimicrobial compounds. Recent study in our group have shown that bacterial inter-specific interactions can be essential for triggering antimicrobial activities in bacteria that did not show any antagonistic activity as monocultures (Garbeva *et al.*, 2011).

We developed a high throughput method for screening bacteria for antimicrobial activities during competitive inter-specific interactions. In total 135 bacterial soil isolates were screened in 2295 unique interactions for the production of antimicrobials during one-to-one confrontations in various combinations. Antimicrobial activity was recorded via an agar overlay assay against two pathogenic target organisms *E. coli* WA321 and *S. aureus* 533R4.

Different pairs of soil isolates that showed antimicrobial activity only during inter-specific interactions but not in monocultures were discovered like *Burkholderia* sp. and *Paenibacillus* sp., *Janthinobacterium* sp. and *Xanthomonas* sp. and many others. The identification of the antimicrobial compounds that are produced during these inter-specific bacterial interactions and the mechanism of interaction are under current investigation.

References: Garbeva *et al.*, (2011) ISME Journal 5:973-985

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The remarkable capacity of *Burkholderia* spp. to interact with soil fungi

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Bacteria and fungi comprise considerable parts of the total biomass in soils and are main contributors to soil life support functions. In the light of the potential of soil life forms to mutualize, we surmised that studying the putative interactions between these groups is important. In recent studies in our laboratory as well as in that of others, evidence has emerged for the specific capability of specific soil bacteria, in particular members of the genus *Burkholderia*, to (actively) interact with soil fungi of the genera *Laccaria*, *Lyophyllum* and others. A main driver of such interactions is the need of these heterotrophic soil bacteria to efficiently acquire carbon sources that are made available at hot spots established by such soil fungi. The

achievements so far obtained from our studies will be discussed, as well as the potential future avenues to explore this emerging area of research. Briefly, in a range of experiments with soil fungi, we took as criteria (1) the prevalence of particular bacterial types in the mycospheres of (mycorrhizal) fungi in the field and (2) the capacity of such bacteria to migrate with developing fungal hyphae through soil. Consumption of glycerol released by the host fungus *Lyophyllum* sp. strain Karsten was a main driver of several bacteria, including a *B. terrae* strain denoted BS001. Furthermore, particular bacterial systems, i.e. the type three secretion system, migratory capability and biofilm formation, may be involved in the colonization of fungal hyphae. Moreover, the pH surrounding fungal hyphae was modulated to support the survival of fungal-associated bacteria. Evidence for an effect of strain BS001 on fungal physiology, notably the inhibition of primordium setting, was also found. Finally, *Burkholderia terrae, B. phytofirmans* and *B. xenovorans* - like types were proficient colonizers of the *L.* sp strain Karsten hyphae. We sequenced the genomes of five such fungal-interactive - next to two plant-interactive - *Burkholderia* sp. strains and analyzed their gene content. In summary, different bacterial capabilities appear to allow members of this genus to find and colonize hospitable niches at soil fungi.

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Growing on the nasty stuff – organohalide respiration unravelled by genomics and proteomics

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Introduction: Organohalide respiration is an unusual form of anaerobic respiration. It is the utilisation of halogenated organics as electron acceptors, which are reductively dehalogenated in turn. This type of respiration is of environmental importance, because of the toxic and persistent nature of many organohalides. Two distinct types of metabolism occur in organohalide-respiring microorganisms: Obligate organohalide-respirers use organohalides as sole electron acceptor as opposed to the versatile type, in which organohalides are only one of many utilised e^--acceptors.

Objectives: The catabolism of organohalide-respiring organisms of both, versatile and obligate organohalide respirers, should be compared using genomics and proteomics. A range of genomes of organohalide-respirers is already sequenced and this battery of sequences was extended with two additional genomes of *Dehalococcoides* strains with an obligate metabolism and genomes of PCE-dechlorinating, versatile *Sulfurospirillum* species. The differences between the genomes and the synthesised proteins should be compared in order to highlight differences between organohalide-respiring organisms.

Methods: Genomes of selected organohalide-respiring bacteria were sequenced using next gen sequencing, annotated genomes were compared with available genomes of organohalide-respiring bacteria. Differential proteomic studies were conducted using a Orbitrap mass spectrometer after culturing bacteria on different organohalides and long-time cultivation without organohalides.

Results: Comparative genomics and differential proteomics in *S. multivorans* led to the discovery of a ~50kbp region coding for enzymes taking part in PCE respiration. As opposed to other organohalide respirers, a putative quinol dehydrogenase was found in *S. multivorans*, which is thought to take part as electron transfer protein in the PCE respiratory chain. *Dehalococcoides* lacks known e^--transport proteins but seems to make use of a membrane-bound complex for organohalide respiration.

Conclusion: The results show that the different physiology of organohalide-respiring bacteria is reflected in the corresponding genomes, especially in gene clusters coding for enzymes responsible for reductive dehalogenation. Further catabolic differences will be highlighted and their role in organohalide respiration discussed.

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Sequence-Inferred Microbial Community Interactions Involving *Metallosphaera yellowstonensis* MK-1

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Genome sequence from isolates and communities from several geothermal springs from Yellowstone National Park (YNP) are now available, providing a unique resource for predicting interactions that occur within these microbial communities. We have curated the annotation of and conducted comparative sequence analysis on *Metallosphaera yellowstonensis* MK-1, an Fe(II)-oxidizing isolate from a YNP acid hot spring with close relatives detectable in metagenomes derived from iron mats that form in multiple low pH geothermal springs. Our analyses revealed extensive evidence of foreign DNA in the genome, including an integrated SV1-like fusellovirus, numerous IS elements (17% of the total predicted proteome), and multiple loci with low GC content, some of which encode proteins having >90% identity to ones found in Sulfolobales sp. that inhabit the same YNP iron mats. Acquired genes encode functions that participate in aromatic hydrocarbon degradation, electron transfer reactions (H_2, nitrate, tetrathionate), toxic metal resistance (Hg, As), and polysaccharide biosynthesis. The MK1 genome (and metagenome assemblies of related strains) lacks genes necessary for synthesis of biotin and nicotinamide, indicating that they are dependent on other members of the community for these essential vitamins. Analysis of six iron mat metagenomes revealed that several taxa were able to produce nicotinamide, but only a single biotin-producing taxon, *Hydrogenobaculum*, was detected. Geoarchaeota, a dominant heterotrophic member of the higher temperature iron mats also does not make either vitamin, but lacks an apparent biotin requirement and thus would not be dependent upon other community members for this cofactor. These observations suggest that YNP *Metallosphaera* and Sulfolobales sp. form intimate associations that enable exchange of DNA. Furthermore, vitamin exchange is likely an important metabolic interaction contributing to the stability of these communities.

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Genotype-phenotype relationship in denitrifying bacteria?

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Question: The relationship between phylogeny and phenotype of denitrifying bacteria is unclear. Current understanding of the biochemistry and physiology of denitrification is based on experiments with a few model organisms. Available sequences of genes encoding denitrification reductases suggest large diversity, but we hypothesize that the phenotype is more dependent on the regulatory network involved than on the phylogeny of the functional genes.

Methods: We investigated the Denitrification Regulatory Phenotypes (DRP) of 8 strains within the genus *Thauera*, belonging to the β-Proteobacteria. These bacteria are common in soils and may reach high abundancies in wastewater treatments. Characterizations were based on quantitative analyses of the accumulation of NO_2^-, NO and N_2O, oxic/anoxic growth and transcription of functional genes. Whole genome sequencing was performed on 6 of the strains.

Results: The results demonstrated a variety of DRP. Four strains were characterized by a rapid, complete onset (RCO) of all denitrification genes and no detectable nitrite accumulation. The others showed progressive onset (PO) of the different denitrification genes. The PO group accumulated nitrite, and no transcription of *nirS* (encoding nitrite reductase) was detected until all available NO_3^- (2 mM) was consumed. All eight strains controlled NO at nano-molar concentrations, possibly reflecting the importance of strict control of NO for survival. Transient N_2O accumulation differed by two orders of magnitude between the strains, indicating that control of N_2O is less essential for survival.

The phenotypes within *Thauera* are profoundly different regarding their impact on their own habitat (nitrite accumulation) and the global environment (N_2O emission). No congruence was seen between these phenotypic characteristics and the phylogeny (16S rRNA and functional genes) and DRP.

Conclusions: The results suggest that the promoter regions and the regulatory network may be more interesting than the functional genes when searching for genetic characteristics of phenotypes such as PO versus RCO. A consequence of this is that quantification and sequence analyses of regulatory genes involved in denitrification may also provide more information than the functional genes themselves for predictions of NO and N_2O emissions from complex systems such as soils or wastewaters.

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The plant microbiome – new perspectives for biocontrol and growth promotion

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New generation sequencing techniques have significantly improved our understanding of the structure and specificity of the plant microbiome. Although microbial inoculants containing biocontrol agents and plant growth promoting rhizobacteria (PGPRs) have been already developed, the importance of the plant's microbiome in the context of plant health and growth are largely unexplored. Millions of microbes inhabit plants, forming a complex ecological community that influences plant growth and health through its collective metabolic activities and host interactions. Viewing the microbiota from an ecological perspective can provide insight into how to promote plant health and stress tolerance of their hosts or how to adapt to a changing climate by targeting this microbial community. In this round table we will discuss i) the current state of microbial inoculants, ii) new results on function and structure of the plant microbiome and, iii) strategies to support beneficial organisms as well as innovative treatments on plants.

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When worlds collide – the biosphere meets the geosphere in heavy oil reservoirs

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Question: Heavy oil deposits are one of the most dramatic manifestations of microbial activity on the planet. The vast oil sands accumulations in Northern Alberta and Venezuela and smaller occurrences of heavy oil worldwide, have resulted from the slow in-reservoir degradation of crude oil over geological timescales. Over a number of years we have been asking what processes have led to the formation of the world's heavy oil and what factors control the occurrence of biodegraded heavy oil.

Methods: We have combined laboratory and field studies of the microbial communities present in heavy oil reservoirs with geochemical analysis and modelling to better understand the drivers and controls on heavy oil formation.

Results: Our interdisciplinary approach has demonstrated that methanogenic degradation of crude oil hydrocarbons is a major process leading to heavy oil in low temperature (<80°C) oil reservoirs where sulfate is limited. A subgroup of the genus *Smithella* is an important organism in many methanogenic oil-degrading systems but may not always be significant players in petroleum reservoirs.

Conclusions: Methanogenic degradation of crude oil is a central process in the development of heavy oil deposits. This opens up possibilities for recovery of energy from stranded oil by conversion of a proportion of this to methane which can be recovered more effectively than oil. Electricity generation from methane produces less CO_2 per KWh than oil or coal and so may also be a route to lower emission energy recovery from fossil fuels.

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Identification of a plasmid encoded novel amidase that catalyzes the first step in degradation of the groundwater pollutant 2,6-dichlorobenzamide (BAM) in *Aminobacter* sp. MSH1.

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Introduction: 2,6-dichlorobenzamide (BAM) is a recalcitrant pesticide metabolite and a major groundwater pollutant in Europe. *Aminobacter* sp. MSH1 is a bacterium that is unique in its ability to mineralize BAM through the metabolite 2,6-dichlorobenzoic acid (DCBA) but nothing is known about the genes and proteins involved.

Objectives: Identification and characterization of genes involved in BAM degradation in *Aminobacter* sp. MSH1.

Material & methods: Plasmids were isolated by alkaline lysis and phenol/chloroform purification. Fractionation of a crude protein extract of *Aminobacter* sp. MSH1 was done by ammonium sulphate precipitation and subsequent Anion Exchange Chromatography. Trypsin digested proteins fractions were sequenced using LC-ESI-MS/MS. Draft genome and plasmid sequences were obtained by Illumina sequencing.

Results: The enzyme catalyzing the conversion of BAM to DCBA was purified by fractionation of a crude protein extract of *Aminobacter* sp. MSH1 and sequenced using LC-ESI-MS/MS. The corresponding ORF (*bbdA)* was identified in the draft genome sequence of strain MSH1 and its activity was confirmed by heterologous expression in *E. coli*. BbdA shows only limited similarity to other known proteins. The most similar enzyme is a 2-fenylpropionamide amidase identified in *Agrobacterium tumefaciens*. The predicted structure of BbdA shows the presence of a rare N-terminal α-helical domain involved in forming a narrow substrate binding tunnel. Strain MSH1 contains around 5 large plasmids. The loss of the capability to degrade BAM in spontaneous mutants corresponded to the loss of one plasmid. Sequencing of the plasmids confirmed that *bbd*A is plasmid encoded. The plasmid is an IncP-1β plasmid (pBAM1) that lacks a part of the mating pair formation *trb* genes and whose backbone showed a genetic organization similar to this of plasmid pEST4011 although pEST4011 is an IncP-1δ plasmid.

Conclusions: A novel amidase, catalysing the conversion of BAM to DCBA was identified and its activity confirmed by heterologous expression in *E. coli*. *bbdA* is encoded on an unusual IncP-1β plasmid.

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Mobilomic analysis suggests an important role of mobile genetic elements in bacterial adaptation towards pesticide degradation in on-farm biopurification systems

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Introduction: On-farm biopurification systems (BPS) are used to treat pesticide contaminated waste water. BPS receive pesticides at relatively high concentrations during a substantial time period of the year, thus perceiving strong and long-term selective pressures for evolution and growth of pesticide degrading bacteria. Plasmids and other mobile genetic elements (MGEs) collectively referred to as the mobilome, and in particular IncP-1 plasmids and IS*1071* elements, have been proposed to be important mediators in the genetic adaptation of bacteria towards pollutant biodegradation. This hypothesis was examined by a metagenomic exploration of the occurrence of IncP-1 and IS*1071* elements as well as their accessory genes in BPS.

Objectives: Unravel the ecological significance of IS*1071* and IncP-1 as adaptive agents in bacterial communities of pesticide polluted environments.

Material & Methods: PCR and qPCR based detection of genetic markers for MGEs and catabolic genes were performed. Catabolic activities were examined by mineralization of ^{14}C-labeled pesticides and degradation of chloroaromatic compounds. Longe range (LR) PCR methods directed toward amplification of accessory genes of IS*1071* composite transposons or IncP-1 plasmids were developed and performed on metagenomic DNA from microcosm BPS and BPS in operation. LR amplicons were sequenced using Illumina.

Results: High prevalence of as well IncP-1 plasmids as *IS1071* elements were observed in pesticide treated microcosm simulating the BPS filter matrix and in BPS in operation at farms, with concomitant increase in the catabolic capacity for pesticide and haloaromatic degradation and in the abundance of catabolic genes compared to non-treated systems. The LR PCR approach generated amplicons up to 33kb, with detection limits of 10^5 copies per gram of soil. The method was successfully applied on DNA extracts from a microcosm BPS and a BPS in operation. Next generation sequencing of the amplified DNA revealed more than 350 kb of unique DNA that was highly enriched in coding sequences for organic xenobiotic catabolism including dioxygenases and dehalogenases.

Conclusion: Our data show the remarkable catabolic content of microbiota in a BPS at the genetic level and suggest that the mobilome is an important mediator in shaping this genetic content.

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Functional gene responses in a perfect world – Can we trust quantification of transcripts in soil in response to manmade chemicals?

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We are all struggling with it: Analyzing environmental relevant microbial processes in situ is extremely challenging. Nucleic acid based studies are becoming more and more popular, but pitfalls exist and should be taken into account. In a perfect world we would know the genes involved, we would know the expression pattern, we would know the mRNA degradation rate, we would know the nucleic acid extraction biases, we would trust our DNase treatment, reverse transcriptase reaction and the quantitative PCR. Does the perfect world exist?

In a less perfect world the functional genes might not be known - or at least only some of them are known. Such a scenario might be when searching for *tfd*A degradation genes for modern herbicides in a soil originating from below a burial mound that has been isolated from the surrounding environment for more than 5000 year.

Another less perfect world would be RNA/DNA extraction from inorganic clay sediments - in this scenario the problem is that when cells are lysed in the nucleic acid extraction procedure the nucleic acids sticks to the clay due to the phosphate backbone. However, using an optimized RNA/DNA extraction protocol including the patented G2 blocking solution, we were able to obtain high-resolution expression profiles of the functional reductive dehalogenase genes *bvcA* and *vcrA* during two consecutive dechlorination events of trichlorethene (TCE), *cis*-dichlorethene (*cis*-DCE) and vinyl chloride (VC) in a clay subsurface environment. Up-regulation of the *bvcA* (for the biostimulated microcosms) and *vcrA* (for the bioaugmented microcosms) gene expression fitted well with high rates of dechlorination of VC, while no known transcripts could be measured during TCE and *cis*-DCE dechlorination. But is this trustable?

What is needed to further establish quantitative transcript based analysis of functional genes in environmental samples? - will we be able to adapt rules for gene expression as used in mammalian cells? Should a housekeeping gene be used for validation? - if so which?

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Spread of tetracycline resistance genes from cow excrements to pasture soils

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Introduction: Regular treatment of farm animals with tetracyclines (TC) leads to the selection of resistant bacteria harboring various tetracycline resistance (TC-r) genes in animal guts, and these genes may be spread to the environment via animal excrements.

Objectives: We aimed to show (i) which TC-r genes are present in excrements of cows that receive chlortetracycline (CTC) as a prophylaxis, (ii) which TC-r genes can persist in pasture soils amended with such cow excrements, (iii) which plasmids are involved in horizontal transfer of TC-r genes between fecal and soil bacteria.

Methods: 22 dairy cows with regular CTC treatment were screened by PCR for the presence of TC-r genes in their excrements. Mixture of excrements from 3 cows was used for soil (n = 3) amendments, either alone or in addition with a low (0.1 mg/kg) or a high (100 mg/kg) dose of CTC, in a microcosm experiment. TC-r genes were assessed by real-time PCR at 0, 14, 28, 58 and 86 days of incubation. Plasmids encoding TC-resistance were exogenously captured from cow excrements and are being further characterized.

Results: All cows harbored tet(O), tet(Q) and tet(W) regardless the current CTC treatment. In excrement-amended soils, tet(O) and tet(Q) dropped under the detection limit after 14-28 days, while tet(W) persisted in all soils for 3 months. CTC at any dose did not increase the gene persistence in soil. Plasmids encoding resistance to TC were isolated from cow excrements, and their preliminary characterization showed the presence of IncP1ε group.

Conclusion: Cows receiving regularly CTC harbored stably at least 3 TC-r genes. Out of them, tet(W) was shown to persist in excrement-amended soils for at least 3 months, indicating that it could have been horizontally transferred to soil bacteria. IncP1ε plasmids might be involved in the transfer of tetracycline resistance from cow excrements to soil.

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Antibiotic resistance associated with waste water treatment plant effluent

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Waste water treatment plants (WWTPs) receive complex mixtures of chemicals and bacteria from multiple origins, creating hotspots for selection and transfer of adaptive genes. Studies have shown that genes for various antibiotic resistance phenotypes persist in sewage sludge applied to land. Less is known about the impacts of liquid effluent, which may introduce rivers with antibiotic resistance genes and antibiotic residues resulting in reservoirs of antibiotic resistance that could disseminate throughout aquatic systems. $Bla_{CTX\text{-}M\text{-}15}$ is the primary cause of third generation cephalosporin resistance worldwide. Understanding the dissemination of key antibiotic resistant genes such as $bla_{CTX\text{-}M\text{-}15}$ is vital to halting the spread of multi-resistant *Enterobacteriaceae.* The dissemination of $bla_{CTX\text{-}M\text{-}15}$ is poorly understood with evidence suggesting multiple transfer events could

have occurred. Despite this poor understanding, few studies have looked at *bla*$_{CTX-M-15}$ carriage and transfer in the wider environment.

In this study sediment samples were taken upstream and downstream of a WWTP outfall to analyse the impact on the microbial resistome. Third generation cephalosporin resistant *Enterobacteriaceae* were found in significantly higher numbers downstream compared to upstream. Isolates were characterised and found to carry *bla*$_{CTX-M-15}$. Analysis of the flanking regions of *bla*$_{CTX-M-15}$ revealed many novel mechanisms of carriage, with more diversity of carriage described in this study, than in the entire clinical world combined. There was evidence for the recombination and transposition of insertion sequence elements, which formed large pathogenicity regions of resistance and toxin genes. Many of these new genetic environments were found solely downstream of effluent. Plasmid analysis revealed multiple plasmid types carried *bla*$_{CTX-M-15}$ and 96% of isolates had the ability to transfer *bla*$_{CTX-M-15}$. *Bla*$_{CTX-M-15}$ *was* recovered in human pathogens such as *Escherichia coli* ST131and *Klebsiella oxytoca,* which as revealed by risk modelling, is a significant threat to human health.

This data suggests that CTX-M-15 mobilisation may primarily occur in the environment, and that WWTP effluent may drive the formation of environmental reservoirs of CTX-M-15, posing a risk to human health in the process.

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Escherichia coli: – a model for monitoring antibiotic resistance spread into the environment and quantification of resistance genes in wastewater treatment plants

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The phenomenon of antibiotic resistance started to be considered a worldwide threat after that antimicrobial resistances were detected in clinically relevant bacteria, leading to an increase of morbidity and mortality within hospitalized patients. Recently, relevant amounts of antibiotic resistances have been found in environmental microbial communities rising the concern for a potential reintroduction of multiple resistant microorganisms into human body especially trough aquatic ecosystems.

Due to the increased risk of antibiotic resistance spread and potential reintroduction of multiple resistant microorganisms into human body.

Wastewater treatment plants are considered one of the relevant anthropic ecosystems which are bridging the gap between human and environmental ecosystems and where the increased risk of antibiotic resistance spread may occur due to the favorable condition for bacteria to grow and exchange genes.

E.coli were isolated from raw and hygienized waters of the Dresden-Kaditz municipal wastewater treatment plant (Germany). Raw waters were collected from sewage pipes which are connecting the two most populated neighborhoods of the city to the wastewater treatment plant. The isolated *E.coli* were tested for their sensitivity to antibiotics and genotypically characterized.

The aim of this work was to compare two inflows to the wastewater treatment plant, with the outflow after the hygienization treatment. In order to investigate if the outflow samples are carrying more multiple antibiotic resistant strains and to find out if the two suburbs give a different contribute of resistant microorganisms to the wastewater treatment plant.

In this work 200 isolates of *E.coli* were tested for resistance against 20 antibiotics. Mainly all *E.coli* isolated from raw and treated water were resistant to Piperacillin, Cefotaxime, Cephalotin, Kanamycin, Tetracycline, Doxycycline, Ceftazidime and Tobramycin.

Instead of a depletion of resistances after wastewater treatment, the hygienized outflow waters shown an increasing amount of *sul1*, *sul2*, *CTX-M-32* genes into the whole wastewater environmental community compared to the inflows waters.

These results suggest that wastewater treatment plants are a platform for selection of antibiotic resistant bacteria during the whole hygienization treatment process and that *E.coli* could be a suitable candidate to monitor the severity level and diversity of antibiotic resistance in urban systems for a majority of antibiotics in use.

48

Advances in microbial ecology – drivers and limitations

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Techniques are frequently suggested as both major drivers of, and limitations to advances in our understanding of interactions between microorganisms and their environments. As an example, the requirement for cultivation was seen as a limitation to microbial community ecology and the application of molecular techniques is seen a providing a significant advance. Certainly, the past two decades have seen an explosion of research into microbial community ecology that has been fuelled by molecular techniques that have generated increasingly detailed and deep descriptions of community composition and, more rarely, of associated activity and ecosystem function. This increase in 'knowledge' of communities has arguably not been matched by an increase in understanding. This proposition will be illustrated and developed by consideration of typical investigations the drivers of community composition and the ways in which it is influences by environmental factors or environmental change. The fundamental ideas on which such studies are based will be discussed and traditional approaches will be critically explored. In addition, the ways in which developments in associated areas, in particular microbial evolution, challenge our traditional ecological ideas will be considered as an example of the requirement for conceptual approaches, rather than merely descriptive research.

P1

Transcriptional noise in natural and synthetic *E.coli* promoters

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Introduction: Many molecules are present in small numbers in the cell. This can lead to large fluctuations in the concentration of these molecules. When these fluctuations ("noise") affect cell growth, selection will act to minimise the level of noise.

Objective: Here we ask to what extent selection has acted to minimise noise in gene expression.

Material and Methods: To address this question, we develop a novel method to produce large numbers of synthetic promoters that that are selected to confer a specific level of expression, but which are not selected based on their noise characteristics. We then compare the level of gene expression noise conferred by these synthetic promoters to that conferred by native *E. coli*.

Results: We show that the synthetic promoter sequences exhibit noise levels as low as the lowest noise levels observed in *E. coli* native promoters. In addition, the majority of native promoter sequences show significantly higher levels of noise than any of the synthetic promoters. These results suggest that a large fraction of native promoters have experienced only weak selection to decrease their noise. Moreover, the existence of a group of native promoter sequences that exhibit high noise suggests that selection may have acted to increase their noise levels.

Conclusions: Using simple theoretical arguments, we show that such selection for increased noise naturally arises whenever gene regulatory systems are too imprecise to ensure that mean expression levels match the desired levels which maximise growth in a given environment.

P2

A *myo*-inositol utilization pathway present on a genomic island contributes to *Aeromonas hydrophila* virulence and the emergence of an epidemic in catfish

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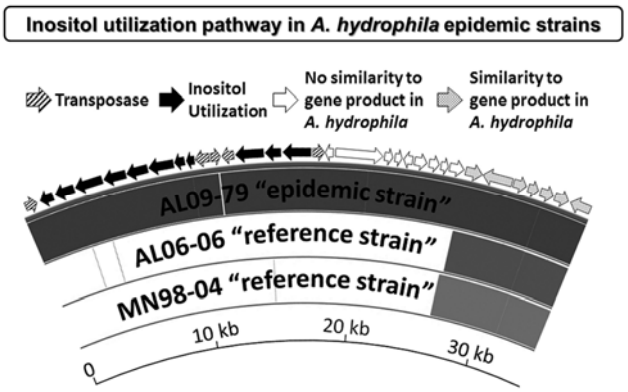
Introduction: *Aeromonas hydrophila* is typically an opportunistic pathogen that can cause Motile Aeromonad Septicemia (MAS) in stressed fish. However, a recent epidemic outbreak of MAS in catfish in the Southeastern United States has caused the loss of over 10 million pounds of fish since 2009.

Objectives: 1) To understand the evolution of this bacterial pathogen by comparing epidemic strain *A. hydrophila* genomes with those of historical *A. hydrophila* isolates, and 2) To test the contribution of epidemic strain-specific genetic loci to the virulence of *A. hydrophila*.

Methods: We sequenced 12 *A. hydrophila* strains, including epidemic and historical isolates, using Illumina sequencing at >100-fold coverage. Bioinformatic analyses were conducted including a pan/core genome analysis, a proteome comparison, and prediction of genomic islands. Bacterial strains were tested for use of *myo*-inositol as a sole carbon source in M9 minimal medium. Allelic exchange was conducted using *A. hydrophila* genes interrupted with a chloramphenicol resistance cassette. Fish disease challenges were conducted by intraperitoneal injection of 10^6 CFU per fingerling catfish.

Results: We identified 54 genomic regions unique to the epidemic strains. Several predicted functions, including a *myo*-inositol utilization pathway, were uniquely present in all epidemic strain genomes. Proteome comparisons indicate that the six epidemic strains share a very high degree of homology (>99%) and only share 65% to 74% homology with reference strains. All epidemic isolates were able to grow on *myo*-inositol as a sole carbon source and this discriminatory phenotype can be used as a diagnostic. An *A. hydrophila* epidemic strain *iolA* mutant was unable to use *myo*-inositol as a sole carbon source and was completely attenuated at a dose that resulted in 93% mortality for its wild-type parent.

Conclusion: We conclude that *A. hydrophila* epidemic strains contain a *myo*-inositol utilization pathway that allows epidemic strains to use *myo*-inositol as a carbon source, is required for the virulence of these strains, and appears to have been introduced via lateral genetic transfer (LGT). These studies suggest that LGT of a *myo*-inositol utilization pathway has directly contributed to the emergence of an epidemic strain of *A. hydrophila* in farmed catfish.



P3

Assessing the permissiveness of complex bacterial communities towards conjugal plasmids – development of a novel method

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Introduction: A crucial parameter governing the significance of horizontal gene transfer (HGT) in a bacterial community is the community's permissiveness. Permissiveness describes the fraction of a microbial community able to receive an introduced plasmid at quantitative and phylogenetic levels. Permissiveness in

complex, natural communities has not been studied intensively, because no suitable methods are available.

Objectives: The purpose of this work was to develop a method for estimating permissiveness of a complex, natural, microbial community towards plasmids that allows to simultaneously:

- Estimate plasmid transfer frequency to the recipient community
- Isolate and taxonomically identify the transconjugants

Methods: Three *mCherry*-tagged donor strains (*P. putida, E. coli* & *Kluyvera spp.)* carrying one of four *gfp*-tagged plasmids (RP4, pKJK5, pIPO2Tet, pRO101) were mixed with a soil bacterial community in a filter mating assay mimicking natural nutrient conditions. Plasmid transfer frequency was quantified by detecting green fluorescent transconjugant microcolonies using stereomicroscopy and image analysis. Transconjugants were isolated using fluorescent activated cell sorting (FACS) with triple gating for bacterial size, *gfp*-based green fluorescence and exclusion of mCherry based red fluorescence. Sorted transconjugants were subsequently analyzed by 454 amplicon pyrosequencing.

Results: Transfer frequency differed depending on plasmid and donor strain. For all combinations with visually detectable transfer, sorting of transconjugants by FACS was possible. Plating of more than 200 sorted transconjugants showed no recovery of red fluorescent donor cells, whereas more than 99.5% of the colonies were *gfp*-positive. The number of sorted cells obtained after FACS was considered high enough for subsequent pyrosequencing for taxonomic analysis of the transconjugal communities.

Conclusions: The combination of filter mating assays, stereomicroscopy and FACS enables us to isolate transconjugants from a complex donor-recipient mixture, and subsequently identify these by pyrosequencing at a phylogenetic level. We can therefore relate the transfer frequency of a plasmid to the fraction and identity of bacteria that actually take part in HGT, thereby determining the permissiveness of the community towards plasmids.

P4
Tracking microbial evolution through CRISPRs

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The microbial world is filled with examples where specific functions (and maybe microbial strains) are critical to specific ecosystems. The main difficulty when addressing this issue is the wide genetic variation among microorganisms. Boundaries between species or OTUs blur into each other in the microbial world and horizontal gene transfer (HGT) continues to transform these boundaries. Viruses play an important role in HGT, and can control population balance in ecosystems and act as selective drivers of evolution. CRISPRs (Clustered Irregularly Interspaced Short Palindromic Repeats) could be defined as the bacterial and archaeal immune system against phage infection. They act by retaining viral sequences (spacers) from past infections. Spacers are transcribed into RNA molecules able to guide Cas proteins to degrade invading viruses containing similar sequences. New spacers are incorporated in the bacterial genome at the leader end of the CRISPR array, and thus, provide a chronology of virus-host interactions. Analysis of CRISPR sequences might not only yield information about short term population dynamics, but could help us design a model for virus-driven evolution in microbial biomes. CRISPR sequences were extracted from metagenomic data of different environments, they were found present in a wide range of ecosystems. Their abundance and composition was highly diverse. Analysis of the conserved gene *Cas1* sequences present in the metagenomes allowed phylogenetic classification of CRISPR systems. Viral identity of the spacers and bacterial identity of the repeated domains was tracked. The results obtained for each environment were then compared. CRISPRs might be excellent tools for monitoring microbial community evolution in environmental samples.

P5
Lysogeny mediates the survival of *Escherichia coli* in marine sediment

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Introduction: The persistence of *E. coli* in habitats external to animal hosts (e.g. sediment) is a widely reported but poorly understood phenomenon. It remains as not known what ecophysiological traits are required for nutrient acquisition and stress resistance in external habitats, and how such traits are acquired. Lysogeny is known to play instrumental roles in *E. coli* genome diversification and subsequent niche expansion from one host to another. Therefore, we hypothesis that lysogeny is a mechanism that enables some *E. coli* strains to expand their niches from animal hosts to external habitats.

Objective: In the study, we investigated the possible impacts of lysogeny on the survival of *E. coli* in marine sediment.

Materials & Methods: We isolated *E. coli* E1140 from marine sediment. Phages induced from E1140 (using mitomycin C) were characterized for diversity and gene content using RAPD-PCR followed by DNA sequencing. Further, the induced phages of E1140 were used to infect *E. coli* E455 isolated from pig feces; as a result, an isogenic lysogen E445L was created. E1140, E455, and E455L were compared for stress resistance, nutrient utilization profile, and population dynamics in seawater and sediment microcosms.

Results: DNA sequences of the phages induced from E1140 had high levels of homology to genes of bacterial fitness factors and genes that are known to be associated with bacterial species indigenous to marine sediment. The infection of E445 by the induced phages of E1140 resulted in the lysogen E445L that had gained at least one prophage whose genes had high homology to a P2 phage. E445 and E445L did not differ in the rate of growth in nutrient media. But the latter was able to use a wider range of nutrients and had extended survival in seawater and marine sediment.

Conclusions: Genes of indigenous marine bacteria were detected in the phages of E1140, suggesting the possibility of phage-mediated horizontal gene transfer between *E. coli* and bacteria living in marine sediment. Lysogenic infection by the induced

phages of E1140 altered the ecophysiological properties of E445, supporting our hypothesis of lysogeny enabling *E. coli* to thrive in external habitats. Whole genome sequencing of E1140, E445 and E445L is currently underway; the results, with respect to the characterization of prophage genes, will be discussed.

P6

Comparative genome-analysis of a non-photosynthetic basal lineage of Cyanobacteria found in both dark and light habitats

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Cyanobacteria played a central role in the 'Great Oxidation event' due to their unique ability to generate oxygen during photosynthesis. The currently accepted dogma is that all cyanobacteria are photosynthetic. However, a basal cyanobacterial lineage, 4C0d-2, has been identified in aphotic (and photic) habitats via culture-independent 16S rRNA gene surveys suggesting the possibility of non-photosynthetic cyanobacteria. To date, no isolates and no genome sequences have been reported for 4C0d-2, so we embarked on a directed search to obtain representative sequences via deep metagenomics. We identified 4C0d-2 phylotypes in both aphotic (koala and human gut) and photic (bioreactor) habitats and obtained five 4C0d-2 draft genomes from the assembly of ~150 Gbp of Illumina data from these habitats. A comparative genomic analysis revealed that all five 4C0d-2 genomes lacked detectable photosynthetic genes. Completeness estimates for the draft genomes based on 111 conserved single copy marker genes indicate that the genomes are >85% complete suggesting that 4C0d-2 is indeed a non-photosynthetic lineage of cyanobacteria. Metabolic reconstruction of the genomes suggest that members of the 4C0d-2 lineage are heterotrophs. It remains to be determined if this basal group are ancestrally non-photosynthetic cyanobacteria or lost their photosynthetic apparatus.

P7

saAFLP and *hin*-region – new approaches for biodiversity and taxonomy investigation, and diagnostics of the genus *Xanthomonas*

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Introduction: The genus *Xanthomonas* consists of the bacteria which are pathogens for a wide range of plant hosts, including the main agriculture plants. The opportune diagnostics of these bacteria in plant material are the main task to prevent the crop losses. Due to the complex taxonomy of this genus until now it was no possible to determine the true relationships between biochemical and genetical properties of *Xanthomonas* species and invaded plant species. That is why the main task is creation of the new approaches and methods to studying, identification and diagnostics of the bacteria of this genus.

Methods: For analysis of the type and typical strains of species *Xanthomonas* species and pathovariants the known methods were used: the analysis of nucleotide sequences of *16S* rRNA, *gyrB, Xcc0006, Xcc0007* genes and ITS and *Xcc0006-0007* intergenic regions; and new suggested analysis saAFLP with the endonucleases of restriction *Xma*JI and *Xba*I, and analysis of nucleotide seuences of *hin*-region.

Results: The new universal for genus *Xanthomonas* primer systems for PCR and sequencing analysis were designed. By these primers the nucleotide sequences of *gyrB* and *Xcc0006-0007* regions were determined for the type and typical strains. Also we modified the saAFLP analysis for the genus *Xanthomonas*. Using saAFLP analysis the new taxonomic maker, *hin*-region, had been revealed and suggested for the *Xanthomonas* spp..

Conclusion: Based on the analysis of *16S* rRNA and ITS nucleotide sequences of type strain and the data found in GenBank (NCBI) the true relationships for intra-species level were not revealed. *GyrB* gene and *Xcc0006-0007* region allowed to investigate the biodiversity beetween species reliably. The analysis saAFLP was in a good agreement with the results obtained earlier by us and by another scientists. This method allowed to reveal the biodiversity at the inter-species level. All strains had its own patterns, saAFLP patterns were species- and pathovariant-specific pattens. The unique as well as the common DNA fragments were found for every strain. We determine the nucleotide sequence of the common DNA fragment for investigated strains and named it as *hin*-region**.** This region is located between tRNA (Glu) genes, and probably is the promoter region for these genes, and its taxonomy reflects the taxonomy revealed by house-keeping genes and core genome**.**

P8

Distribution and evolution of aromatic degrading genes in Alphaproteobacteria

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Alphaproteobacteria show a great versatility in adaption to a broad range of environments and lifestyles, spanning from relatively unspecific association (as rhizospheric or endophytic strains) to the highly species-specific interaction of rhizobia. In biodegradation of aromatics the genus *Pseudomonas* and *Burkholderia*, from gamma and betaproteobacteria respectively are best studied. The less studied class Alphaproteobacteria harbors a miscellaneous set of metabolisms, cellular phenotypes and occurs in a wide range of habitats, as phototrophs, symbionts of plants, animal and plant pathogens [Pini F, et al., (2011)]; and is also capable of metabolizing C1 compounds. Further Sphingomonas type of bacteria are well suited for living in hydrocarbon polluted environments and thus it's of great interest to study the flexibility in Alphaproteobacterial genomes and how they are prepared for living in contaminated environments.

The aim of the study was to evaluate the importance of phylogeny versus habitat while considering the catabolic potential of Alphaproteobacteria and the distribution of catabolic genes in chromosomes and plasmids in relation evolution of catabolic pathways. Published genomes (140) of Alphaproteobacteria class were selected for the study of the catabolic gene distribution in plasmids, chromosomes or chromids and their movement

within/across the phylum. ClustalW and Mauve tool were used for the genome comparison.

Within the set of Alphaproteobacterial genomes those isolated from the rhizosphere possessed more catabolic genes compared to those isolated from other habitats. *Methylobacterium extorquens* contained open reading frames with significant similarity to genes involved in plant association in *Rhizobia* and *Agrobacterium.* The plasmids in Alphaproteobacteria contained more dioxygenase genes than plasmids in other proteobacteria (Fig 1).

The presence of catabolic genes in mobile genetic element like plasmids in alphaproteobacteria shows the importance of horizontal gene transfer in the evolution of aromatic catabolic pathways in this bacterial class.

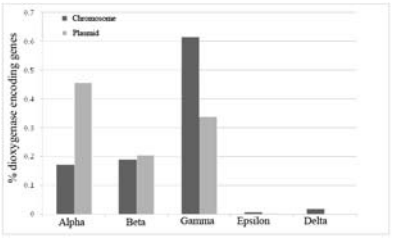

Fig 1: Per cent of dioxygenase-encoding genes per total number of genes encoded in chromosomes and plasmids of Proteobacterial class.

P9

Genomics of a unintrogressed *Campylobacter coli* clade 3 strain

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Introduction: Of the 32 *Campylobacter* species, *C. jejuni* and *C. coli* are mainly associated with human gastrointestinal disease. Population structure-wise, *C. jejuni* genotype clusters are tightly interlinked with host-genotype associations. *C. coli* shows a clade-like structure; clade 1 strains are associated with agriculture and introgression with *C. jejuni* and unintrogressed *C. coli* strains are in clades 2 and 3.

Objectives: A clinical *Campylobacter coli* isolate (76339) was characterized with a unique multilocus sequence type with 4 novel alleles (ST-5088). It also produced gamma-glutamyltranspeptidase (GGT), a characteristic of *C. jejuni* but not described in *C. coli* before.

Methods: The whole genome was sequenced using Roche 454 sequencing and was assembled with the Newbler assembler. The scaffold was verified by PCR. Preliminary annotation was performed on the automated RAST annotation server, followed by manual inspection.

Results: Phylogenomic analysis showed that *C. coli* 76339 belonged to clade 3 of unintrogressed *C. coli* strains. The *ggt* gene was found among both *C. coli* clades 2 and 3 and Bayesian inference of ε-proteobacteria *ggt* orthologs reveals these potential scenarios a) the *ggt* gene was acquired by an ancestral *Campylobacter* species, initially originating from an ancestral *Helicobacter* species and b) during evolution of both *C. jejuni* and *C. coli* the *ggt* gene underwent progressive extinction. Also, *C. coli* 76339 carried a lipooligosaccharide (LOS) locus containing a phylogenetically distinct sialyltransferase not present in clade 1. Chromatographic analysis confirmed presence of sialic acid in the LOS of 76339. Moreover, we identified several features which characterized unintrogressed *C. coli* belonging to clades 3 and 2.

Conclusion: Genomic analysis of C. coli 76339 improved our understanding of the evolution of this species. We propose a novel scenario for the evolution of the accessory *ggt* gene in both *C. coli* and *C. jejuni* species. Finally, the presence of sialic acid on *C. coli* LOS may indicate a possible role of this species in post-infectious neuropathies.

P10

Genomic analysis of anaerobic haloalkaliphilic chitinolytic bacterium "*Chitinivibrio alkaliphilus*" ACht1, a first cultured representative of the candidate phylum TG3

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A novel bacterium "*Chitinivibrio alkaliphilus*" ACht1 growing at pH 10 and salinity up to 3.5 M was isolated from hypersaline soda lake (Sorokin et al., 2012, Extremophiles, 16:883-894). The isolate grows exclusively on insoluble chitin by fermentation. According to the 16S rRNA analysis, ACht1 and several related isolates belong to the candidate phylum TG3 (Termite Group 3), representing its first culturable members. To better understand the biology and the evolutionary history of this novel bacterial lineage, in this work we sequenced the 2,6 Mb genome of the type strain ACht1. The genome analysis revealed enzymes of chitin-degradation pathways, including secreted cell-bound endochitinases, and enzymes involved in hydrolysis of some other polysaccharides. The reconstructed central metabolism revealed the Embden-Meyerhof and pentose phosphate pathways enabling the fermentation of polysaccharides, while it lacks the genes needed for aerobic or anaerobic respiration. Only Rnf-type complex and oxaloacetate decarboxylase were identified among putative membrane-bound primary ion pumps, the generated ion gradient might be exploited for ATP synthesis by sodium-transporting V-type ATP synthase. Phylogenetic analysis using the ribosomal proteins and the taxonomic distribution analysis of whole proteome supported the phylum-level classification of ACht1. This work was supported by RFBR grant 12-04-31945.

P11

Finding specific ecological adaptations of bacterial species: a quest for a renewed taxonomic description of bacteria

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For taxonomy as well as for applied bacteriology, the description of a novel species of bacterium requires the discovery of phenotypic traits that allow its facile identification in any laboratory. Finding these specific traits is generally obtained by chance by screening numerous properties through the use of biochemical galleries for instance. However, this task may be hard to achieve in species complex consisted of closely related species displaying very similar biochemical traits combined with large inter-strain differences within species. Instead of discovering species specific phenotypes by chance, we propose to look for "species specific" properties by using comparative genomics, reverse genetics and genome driven functional ecology. By using a large set of sequenced genomes of the *Rhizobium/Agrobacterium* group (43 *Agrobacterium*, 21 closely related *Rhizobium*), this "reverse ecology" approach allowed us to uncover cryptic ecological adaptations of species that were likely involved in their speciation. Species specific adaptations may be relevant functions to manage bacterial taxa in the environment as for instance controlling the crown gall disease caused by agrobacteria. Moreover, this approach makes possible the association of an "ecological annotation" to bacterial taxa. In our opinion, this should lead taxonomic committees to now request the complete genome sequencings of several markedly different strains for the valid description of novel species in order to add an ecological dimension to formal description of bacterial species.

P12
Inspecting genome histories in *A. tumefaciens* to reveal the role of adaptation in bacterial cladogenesis

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Question: The role of adaptation vs. neutral processes in bacterial cladogenesis is debated. We used the *Agrobacterium tumefaciens* species complex, a diverse group of plant-associated bacteria as a model to search for genomic signatures of adaptation in relation to diversification.

Methods: We reconstructed the evolutionary history of genomes of a dataset of 47 Rhizobiaceae including 22 *A. tumefaciens* using a new phylogenetic approach for reconstruction of ancestral genomes, accounting for events of horizontal gene transfer and duplication. This approach identifies groups of co-transferred/duplicated genes, providing better confidence and accuracy when mapping the event to clade ancestors. Modifications of gene repertoires along species phylogeny may reflect the acquisition of functions involved in ecological adaptation, or be the result of a random gene flow. The combination of functions acquired by transfer of a random segment of genome must rarely encode coherent pathways, but selection may act to retain those rare events when constituting an advantageous new function. We designed a test of neutrality of transfers by computing the degree of functional homogeneity of genes within each transferred block using GO functional annotations and compared them to a null expectation where comparable genome segments are randomly transferred.

Results: Blocks of transferred genes showed a bi-modal distribution of functional homogeneities, some being more homogeneous in their functions than expected, suggesting that selection participates in shaping gene repertoires. We identified blocks of co-transferred genes encoding complete pathways, such as chemotaxis regulation, production of extracellular secondary metabolites or catabolism of plant-derived compounds.

Conclusions: These results suggest that cladogenesis in *A. tumefaciens* may have been driven by ecological adaptations related to interaction with a host plant and competition between rhizospheric bacteria. These data - gene trees, transfer and duplication events, blocks of co-evolved genes, syntenies, functional annotations... - are compiled in an integrative database, Agrogenom, which can be visualized and queried through an interactive web interface.

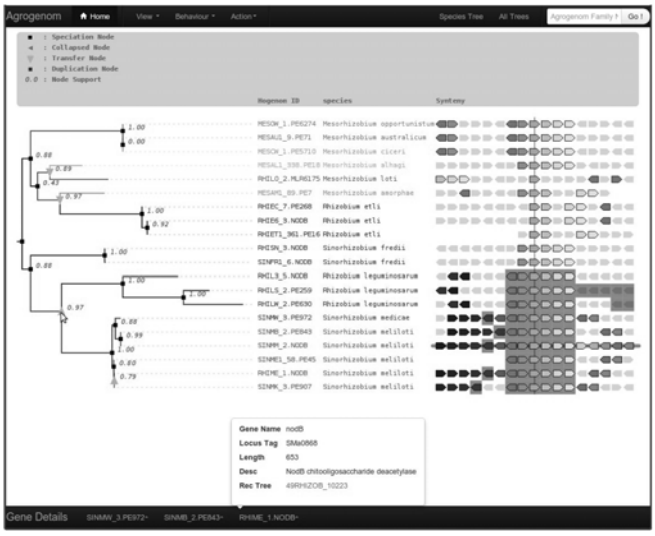

Figure 1: view of the Agrogenom web interface, highlighting a block tranfer event.

P13
***Aeromonas* spp. – Ubiquitous or specialized bugs?**

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The genus *Aeromonas* comprises ubiquitous bacteria which are known to play several roles in the environment. They are mainly known as important disease-causing pathogens of several aquatic organisms, and today they are also considered emerging pathogens associated with a variety of human infections. However, their effective role in human pathogenicity is not clear as a deep knowledge of the genus poses three main controversial issues. First, the *Aeromonas* taxonomy is debated because inter- and intra-species relationships are difficult to characterise, the virulence mechanisms towards humans remain unclear and the evolution of the genus and its adaptation to a variety of environments were not analysed in depth. The possibility to consider these three aspects at the same time and to link genetic information to environmental data could lead to a deeper knowledge of the *Aeromonas* genus evolution, as well as the understanding of potential virulence characteristics. Are there any differences among strains of different origin, and is the virulence pattern a result of the adaptation to specific hosts or environments?

In the present work, we explore the distribution of the *Aeromonas* species across three main hosts: fish and aquatic environment, food and human cases of disease. A Multilocus Sequence Typing (MLST) technique was developed to construct the phylogeny and evaluate the relationships among 258 *Aeromonas* strains. The first *Aeromonas* MLST on-line database was opened (www.pubmlst.org/aeromonas) and it is available for collecting information about *Aeromonas* spp. from laboratories all over the world. The mode of evolution and the adaptation mechanisms were investigated through several software to find a connection between species, host and specific virulence targets. The phylogeny and the allele frequencies were used as inputs to predictive models which reveal the presence of subpopulations and 'habitats' among strains sharing common characteristics.

All the strains were characterised, demonstrating the exceptionally high genetic variability of the *Aeromonas* genus. Two distinct habitats were detected, revealing a different species distribution among the sources (aquatic and terrestrial ecosystems) and the occurrence of adaptation processes of the *Aeromonas* species towards some specific habitats.

P14
Genome sequence of the arsenite oxidizing strain *Aliihoeflea* sp. 2WW

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Introduction: Bacterial transformation of arsenic has a high impact on the arsenic cycle in soil and water, threatening public health in both developing and industrial countries. Biological oxidation of the more mobile and toxic arsenite [As(III)] to the less toxic arsenate [As(V)] helps to alleviate this problem, due to subsequent adsorption of As(V) to metal oxides in ground waters.

Here we report the draft genome sequence and annotation of *Aliihoeflea* sp. strain 2WW, the first reported As(III) oxidizing bacterium belonging to the genus *Aliihoeflea*.

Methods: Strain 2WW was isolated from a biofilter treating arsenic contaminated groundwater in the Northern part of Italy (Cremona, Lombardia). The genome sequence of *Aliihoeflea* sp. strain 2WW was obtained by Illumina paired-end and mate-pair sequencing.

Results: *Aliihoeflea* sp. strain 2WW oxidized As(III) completely to As(V) in the early exponential growth phase within 24 h at 30°C, 96 h at 15° C and within 350 h at 5°C.

The draft genome was assembled into 273 contigs with a genome size of 4,122,965 bp and it was assembled in 10 scaffolds. It had a GC content of 64.37%, 4,139 putative coding sequences (CDS). Genome analysis indicated that *Aliihoeflea* sp. strain 2WW possessed two different genetic islands: one including 4 genes of the *ars* operon for arsenic resistance (*arsR* for regulatory protein, *ACR3(2)* for As(III) efflux pump, *arsC* for As(V) reductase and *arsH* for protein conferring high resistance to As(V)); the second containing an As(III) oxidase *aioBA*. Although strain 2WW possessed *arsC*, it was not able to reduce As(V) to As(III). In addition, many genes encoding putative metal (lead, cadmium, zinc, mercury and copper) transporters and cobalt-zinc-cadmium resistance protein (CzcD) were also identified in the genome.

Conclusions: These results indicate that *Aliihoeflea* sp. strain 2WW is an efficient psychrotolerant As(III) oxidizer that could be used in combination with iron-based materials for arsenic adsorption and removal from contaminated groundwater. The draft genome of strain 2WW provides information to explore arsenic as well as multiple metal resistance mechanisms. The draft genome sequence of strain 2WW has been deposited at GOLD database under the identification code Gi21948.

Acknowledgments

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P15
Replicon-dependent bacterial genome evolution in *Sinorhizobium meliloti*

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Many bacterial species, such as the alphaproteobacterium *Sinorhizobium meliloti,* are characterized by open pangenomes and strains contain multipartite genomes consisting of a chromosome and other large-sized replicons, such as chromids, megaplasmids and plasmids.

The evolutionary forces that shape the pangenome of species with multipartite genomes in both functional and structural aspects are still poorly understood. To shed light on this topic, we sequenced the genomes of ten *S. meliloti* strains, which were combined in our analysis to four publicly-available additional genomic sequences.

Results obtained indicated that the three main replicons present in these strains (a chromosome, a chromid and a megaplasmid) show a partly independent history during strains differentiation. In particular, the chromid was shown to be a hotspot for positively-selected genes and, unexpectedly, genes resident in the chromid were also found to be more widespread in distant taxa than those located in the other replicons. Moreover, through the exploitation of a DNA proximity network, a series of conserved "DNA backbones" were found to shape the structural evolution of the genome, with the rest of the genome experiencing rearrangements.

The presented data allow to depict a scenario where the evolution of the *S. meliloti* pangenome includes a structurally-conserved genome fraction that evolves by positive selection (mainly on the chromid), and a highly-variable fraction, that mostly contributes to structural fluidity and to the emergence of new functions (the megaplasmid), then suggesting that the

chromids could have had a distinctive role in intra-species differentiation.

P16

Promotion of mutation by extracellular nucleic acids in *Escherichia coli*

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Question: Extracellular nucleic acids in the natural environment are source of nutrients and gene pools for bacteria. Bacterial DNA and RNA are mainly released by viral lysis and grazing. Some bacteria actively release naked DNA and RNA into the environment. The amount of extracellular DNA concentration is up to 25-fold higher than that of the DNA contained in all the bacteria inhabiting the given environment. The amount of extracellular RNA constitutes some 10% of that of the extracellular DNA. Extracellular nucleic acids are rich in the natural environment, but their heredity impact on bacterial cells has been masked. In order to understand the genetic impact of extracellular nucleic acids on bacteria, we examined the response of bacteria to extracellular DNA and RNA.

Methods: After culturing *E. coli* in liquid medium without nucleic acids, the cells were placed on agar plates with or without fragmented DNA, RNA, nucleotide, nucleoside, and monosaccharide. The plates were incubated at 37°C for 1 day. Mutant frequencies to nalidixic acid in the cultured population were determined. Gene expression profile was examined by microarray.

Results: *E. coli* showed 27 and 32- fold increase in mutant frequency in the presence of DNA and RNA, respectively. The viable cell number was not affected by addition of DNA or RNA to agar plate. Neither ribose nor deoxyribose showed any significant change in mutant frequency, but certain nucleotide and nucleoside (adenosine monophosphate, adenosine etc.) increased the mutant frequency. Some oxidoreductase genes were commonly upregulated in the presence of RNA, DNA, adenosine monophosphate, and adenosine. Oxidoreductase was involved in generation of ROS via NADH oxidation. Fluorescent staining with carboxy-H_2DCFDA showed that intracellular reactive oxygen species (ROS) increased in the presence of DNA and RNA, and thus ROS might be involved in the increase in the mutant frequency.

Conclusion: Extracellular nucleic acids led to alter the genomic property of bacteria without relying on gene transfer. Extracellular nucleic acids may play an important role in the rapid evolution of the bacterial genome in nature.

P17

The use of microfluidic devices to understand how different transcriptional responses to external stimuli evolve in natural isolates of *Escherichia coli*

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Introduction: Organisms are continually exposed to changing environmental conditions. It is often advantageous for an organism to change its phenotype in response to such changes in environmental conditions. This phenomenon is called phenotypic plasticity.

Objectives: Here we focus on the ability of *E. coli* to adjust its transcriptional program in response to changes in environmental conditions (i.e. phenotypic plasticity). In order to manipulate environmental conditions, and to understand how transcriptional responses are implemented at the single cell level, we use a microfluidic system that allows precise control over culture conditions and continual monitoring of gene expression and cell growth.

Material & Methods: We use a multiple-input PDMS-based microfluidics device first outlined in a previous publication from Wang et al (2010).

Results: Using this microfluidic device, we show that we can quantitatively track gene expression and growth in single cells for many generations while rapidly changing environmental conditions. We then use this experimental setup to address a simple question: how does phenotypic plasticity evolve over time. Using a collection of *E.* coli strains isolated from the environment, we show that at the single-cell level, the regulation of the *lac* operon differs qualitatively between different *E. coli* strains. We suggest further experiments to develop a mechanistic understanding of how these changes occur.

Conclusion: Here we design and implement a microfluidic device to study transcriptional responses to external stimuli in *E. coli* on single cell level. We show that such responses can change over evolutionary time and outline future work that will allow mechanistic insights into how these changes occur.

References: Wang, P., Robert, L., Pelletier, J., Dang, W. L., Taddei, F., Wright, A., & Jun, S. (2010). Robust Growth of *Escherichia coli*. *Current biology*, *20*(12), 1099-1103.

P18

The capacity for multistability in anaerobic chaos – (eco)systems approach to bacterial and archaeal microbial assemblages in rice fields, animal rumen and biogas reactors

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Anaerobic ecosystems such as rice fields, biogas reactors and animal rumen are central to the everyday human life as sources of food, energy, transport and a means of sustainable management of the environment. The three ecosystem types have been treated separately throughout scientific literature although the basic underlying microbial principles are essentially the same. In this study, 148 clone libraries from all three environments were compiled ($n_{bacteria}$=89; $n_{archaea}$=59; spatial dataset) next to 152 deep sequencing datasets from full scale biogas reactors time series ($n>10^6$ reads; temporal dataset). Results show that bacterial and archaeal anaerobic community assembly space was confined to three and one global centers,

respectively, and the three environmental types exhibited significantly different degrees of immigration and fixation of novel species. The number of detected bacterial and archaeal taxa as a function of sampled environments stabilized for the order, family and genus taxonomic levels, except for the rice-field and biogas reactor bacterial communities. Significant global differences in relative bacterial and archaeal taxonomic composition of the three ecosystems were described. Time series analyses of deep-sequencing bacterial biogas reactor data showed varying rates of community change through time at the level of 97% OTU. Assemblages exhibited crossing trajectories indicating that switches from one assembly state to another already encountered in distant and unrelated biogas reactors took place. The data suggest that anaerobic microbial communities explored a limited space of all possible assemblies in order to provide a stable function. Final analyses showed that the amount of assembly space covered by distinct communities differed significantly, indicating that some of the communities reassembled at significantly different rates than others giving rise to events when two functionally equivalent, distinct, unconnected and distant microbial community structures were not significantly different. This opens a new venue for research whether diversity, its estimates and other descriptive elements used so far in fact matter and contribute to our understanding of microbial communities as self regulating systems.

P19

Transcriptomics of quorum-sensing pathway revealed that transfers of the virulence Ti-plasmid and companion At-plasmid are co-regulated in *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens C58 operates two successive horizontal gene transfers in the course of its interactions with plant-host. Firstly, the bacterial pathogen transfers a part of the tumor inducing (Ti)-plasmid to the plant nuclear genome, where it promotes oncogene expression as well as the production of two opines, agrocinopine and nopaline. *A. tumefaciens* can take advantage of these opines through their assimilation as carbon and nitrogen sources. Secondly, agrocinopine de-repressed the quorum-sensing (QS) pathway which leads to replication and conjugation of the Ti-plasmid, and hence to dissemination of the virulence genes among the plant-tumor colonizing bacterial populations.

In this work, we analyzed, both in presence and in absence of nopaline, the genome-wide transcriptional profiles of a WT C58 strain and of an *accR*-defective mutant which mimics the conditions of an exposure to agrocinopine. Surprisingly this revealed that not only the expression of already known QS-regulated genes but also these of important genes for the conjugation of the companion At-plasmid as well as for the nopaline catabolism were up-regulated in the *accR* background. Further conjugation assays confirmed that horizontal transfers of the two At- and Ti-plasmids were strongly stimulated when the *accR* mutant was used as donor strain. In addition, opines consumption tests also demonstrated that nopaline assimilation was potentiated in the *accR* mutant compared to the WT.

Overall these results bring about new information about control of horizontal gene transfer by opines and quorum-sensing in *A. tumefaciens.* It also highlights a new regulatory interplay between the agrocinopine and nopaline regulons, supporting hereby a novel paradigm for the opine signalling in the *A. tumefaciens* C58-induced plant tumors.

P20

Network analysis reveals the co-occurence patterns of bacterial phylotypes in a long- term phytoremediation field experiment

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Microbial responses in stressed and disturbed ecosystem are increasingly being investigated to assess ecosystem sustainability. Little experimental evidence exists to indicate predictable patterns in microbial community structure or composition during the restoration of such ecosystems. Understanding the co-occurrence patterns of microbial taxa in relation to environmental parameters may help us understand their ecological functions *(1)*. To this end, we performed network analyses on massive pyrotag datasets obtained from a phytoremediation field experiment.

A phytoremediation experiment was conducted with Aspen (*Populus tremula*) and hybrid aspen (*Populus tremula x tremuloides*) clones planted in soil from an accidental oil spill site. Vegetated and un-vegetated, oil-polluted soil samples were collected during 2 years of the experiment and 16S rRNA gene amplicons were sequenced on GS-FLX 454 pyrosequencer. Network analysis was carried out in R environment as described previously *(2)*.

NM-MDS revealed the temporal development patterns of microbial communities in vegetated and un-vegetated oil polluted soil. We observed a distinct shift in microbial communities from an early phase to late phase in the degradation process. The network of bacterial OTUs contained 3 main modules (shown in pink, yellow and blue colors : Fig1.). The members of module 1 were the main groups in early phase (Actinobacteria, TM7, Acidobacteria) whereas in the late phase the Actinobacterial and TM7 populations decreased followed by an increase in Betaproteobacteria, Gammaproteobacteria and Acidobacteria (module 2). Although Actinobacteria and Alphaproteobacteria are well known degraders of petroleum compounds, not much is known about the role of TM7. The main modules can be interpreted as specific ecological niches.

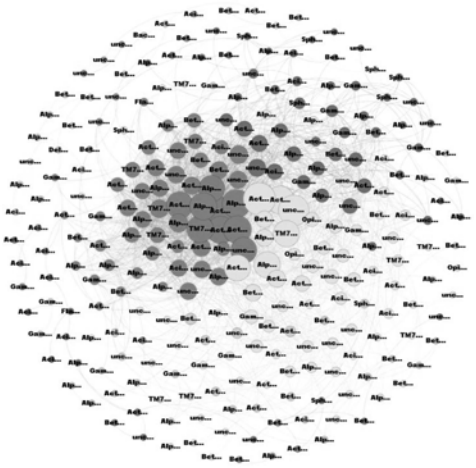

Fig 1: Network of co-occurring OTUs based on correlation analysis. Colours represent the modularity class of nodes (OTUs). A connection stands for a strong(**r>0.6**) and significant correlation. Size of each node is proportional to the number of connections. Abbreviations: Alp -Alphaproteobaceria, Bet- Betaproteobacteria, Gam-Gammaproteobacteria, Act- Actinobacteria, unc- Uncultured.

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2. A. Barberán.*et al. The ISME Journal.* 6, 343-351 (2011).

P21
Structure and sociality of the lettuce core microbiome

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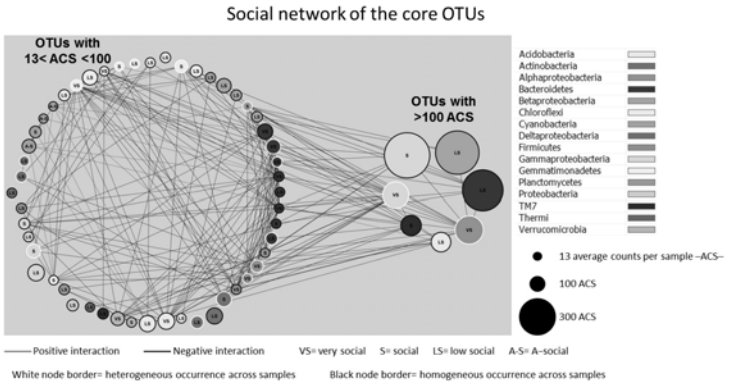
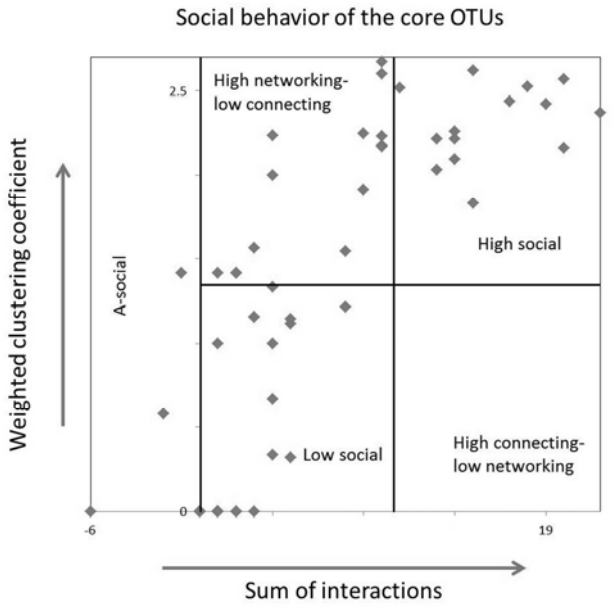
Lettuce (*Lactuca sativa* L.) is highly valued as a leaf vegetable in a health-conscious society, on account of its dietary fibers and vitamins. Despite their ubiquitous presence, lettuce-associated bacterial communities are often neglected in discussions of food safety. We postulate that the native lettuce microbiome can be inhibitory to colonization by pathogenic bacteria. We aimed to (i) study structure of the lettuce core microbiome, (ii) unravel its interaction net, and (iii) identify factors affecting both occurrence patterns and sociality.

The root–associated microbiome of 24 lettuce samples (8 different cultivars, 4 subspecies) and ofthe most closely related wild species *Lactuca serriola* L., was characterized by 454-pyrosequencing of the 16S rRNA genes. Qiime was used to cluster the sequences into 90% similarity OTUs and to compute alpha- and beta-diversity. Occurrence pattern similarity and interactions between core OTUs (occurring in ≥85% of the samples) were assessed by Spearmanρ correlation and tested for statistical significance of OTU taxonomy, abundance and homogeneity. In addition, FISH-CLSM was used to visualize interactions in situ.

The highest host-specificity of the lettuce microbiome was at cultivar level but the core microbiome was more specific at subspecies level. Core microbiome comprised 190 OTUs (17 phyla). Core OTUs were structured into abundance groups. Occurrence patterns were influenced by taxonomy but not by abundance. More than 200 interactions occurred between core OTUs, more than 90% of which were positive (Fig. 1). All negative interactions involved Cyanobacteria; positive interactions were not affected by taxonomy up to the family level; heterogeneous OTUs showed a higher number of interactions. Core OTUs exhibited 4 social behaviors: high social, social (low–connecting but high–networking), low social, a–social (Fig. 2).

The core microbiome of lettuce root system is extremely diverse and mainly composed of low abundant OTUs: these represent a rich reservoir of genetic and functional diversity. Sociality was shaped more by synergism than by antagonism, suggesting long lasting coevolutions within the core microbiota. Taxonomy significantly influenced the occurrence pattern similarity and the interaction type, but not the sociality of the synergistic OTUs.





P22
Mining chitinase-related sequences in mangroves sediments by metagenomics

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The mangrove ecosystems are located in the land-sea interface in tropical and subtropical climates. Therefore, this biome is able to select microbial communities adapted to variations in salinity and able to survive under low oxygen availability. Such environments present high levels of organic matter (about 10%), but low availability of nutrients, mainly due to the recalcitrance of organic matter under anoxic conditions. This study was designed to detect genes involved in the production of degradative enzymes chitinases, exploring samples from four mangrove areas (BrMgv01 to BrMgv04) under distinct levels of contamination. The total numbers of screened sequences were 75,892 from BrMgv01, 197,621 from BrMgv02, 149,207 from BrMgv03 and 146,804 from BrMgv04. Data mining was carried out on the CLC Genomics WorkBench, where blastx was performed contrasting the mangroves databases against chitinase sequences found in public databases (Swiss-Prot; Ref Seq Protein, Protein Data Bank - PDB; nr Protein - NCBI). This approach revealed the constant frequency of chitinase-related sequences around 0,010% (74 in BrMgv01, 186 in BrMgv02, 159 in BrMgv03 and 144 in BrMgv04). The taxonomic affiliation of these sequences indicated the occurrence of such genes in bacterial cells, mainly belonging to the phyla *Proteobacteria* and *Actinobacteria*. In a more detailed view, the prevalent group within Actinobacteria was *Streptomyces*, and for Proteobacteria, sequences affiliated with distinct genera were found in each mangrove. The similarities found between chitinases-related sequences from mangroves and those from databases varied between 39 and 100%. The occurrence of sequences with low similarities with those already described in other environments might indicate mangroves as *hot spots* for chitinase genes, where unique enzymes patterns occur, making such environment highly important in the wider comprehension of biomass degradation and also for biotechnological exploration.

P23
Systematic molecular measurements reveal key microbial populations driving community-wide phenotype

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Natural microbial communities are heterogeneous and dynamic. Therefore, a major consideration for multiple omic data studies is the sample-to-sample heterogeneity, which can lead to inconsistent results if the different biomolecular fractions are obtained from distinct sub-samples. Conversely, systematic omic measurements, i.e. the standardised, reproducible and simultaneous measurement of multiple features from a single undivided sample, result in fully integrable datasets.

Objective: In order to prove the feasibility and benefits of such systematic measurements in the study of the respective contributions of different populations to the community-wide phenotype, we purified and analysed all biomolecular fractions, i.e. DNA, RNA, proteins and metabolites, obtained from a unique undivided sample of lipid accumulating microbial community (LAMC) from wastewater treatment plant and integrate the resulting datasets.

Methods: One time point of particular interest was first selected out of 4 LAMC samples for its high diversity and strong lipid accumulation phenotype. Then, the systematic measurement strategy was applied to the selected undivided LAMC sample and the purified biomolecules were analysed by high-throughput techniques. DNA and RNA sequencing reads were assembled at the population-level using different binning strategies. A database, containing predicted proteins, was constructed to identify the detected peptides. Finally, all biomolecular information was mapped onto the assembled composite genomes to identify the precise roles of the different populations in the community-wide lipid accumulation phenotype.

Results: Metabolomics and 16S diversity analyses were used to select the sample of highest interest for detailed analysis. The systematic measurements of the selected sample followed by data integration have allowed us to probe the functional relevance of the population-level composite genomes, leading to the identification of the LAMC key players.

Conclusion: As community phenotype is not the sum of the different partner phenotypes, understanding a microbial community system requires more than the study of isolated organisms. Even if both approaches are complementary, top-down systematic approached only provides a holistic perspective of micro-ecological processes.

P24
Identification of gene functions involved in synergistic interactions in a pesticide degrading multispecies bacterial consortium

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Introduction: Different bacterial species often live together in consortia which enable them to perform complex tasks such as the degradation of recalcitrant pesticides. An example is the triple-species consortium described by Dejonghe et al. (2003) in which three bacteria synergistically degrade the phenyl urea herbicide linuron. The consortium consists of *Variovorax* sp. WDL1 that converts linuron to 3,4-dichloroaniline (DCA) and *N,O*-dimethylhydroxylamine (*N,O*-DMHA) DCA is degraded by *Comamonas* sp. WDL7 and *N,O*-DMHA by *Hyphomicrobium* sp. WDL6. The consortium forms mixed multispecies biofilms in continuous system fed with linuron. The bacterial features and associated gene functions which determine interspecies interactions for the well-functioning of multispecies bacterial consortia are not well-studied.

Objectives: Determining "interspecies signaling" functions at the genetic level in the linuron degrading triple-species consortium during linuron degradation in biofilms.

Materials and Methods: Draft genomes were obtained by Illumina sequencing. Total RNA was extracted from biofilms using the Promega SV total RNA purification kit. mRNA enrichment was performed using the MICROBExpress kit (Ambion). "Differential fluorescence induction" (DFI) in strain WDL7 is performed using

vector pRU1097 (Karanukaran et al., 2005) containing the promotorless *gfp* gene.

Results: The identification of "inter-species signaling" gene functions is being addressed by two approaches that involve the growth of the consortium as a biofilm in continuous flow chambers and strain WDL7 as target bacterium. The first approach is differential RNAseq between WDL7 grown alone and WDL7 grown in the consortium. To this end, a method was optimized for extracting and enriching mRNA from biofilms in small flow chambers. A second approach is DFI. A library of WDL7 containing the promoter-trapping plasmid pRU1097 was constructed and will be used in biofilm experiments to recognize promoters that are upregulated during growth of WDL7 in the consortium.

Conclusions: Methods were interrogated and developed to identify genes functions that determine interspecies interactions in a linuron degrading consortium.

Reference: Dejonghe, W., et al., *Synergistic degradation of linuron by a bacterial consortium and isolation of a single linuron-degrading Variovorax strain*, Appl. Environ. Microbiol, 2003. **69**: 1532-1541.

Karunakaran, R., et al., *A family of promoter probe vectors incorporating autofluorescent and chromogenic reporter proteins for studying gene expression in Gram-negative bacteria,* Microbiology, 2005. **151**: p. 3249-56.

P25

Crime and punishment of social interactions in *Bacillus subtilis*

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In a social process called quorum sensing (QS), bacteria secrete and bind signalling molecules with signal specific receptors thereby sharing the information on the surrounding environment. It is not known how occurrence of signal deficient mutants affects this information flow. Here, through use of the single cell fluorescence microscopy we employed QS system of *Bacillus subtilis* as a model to explore the response of both, mutants and the wild type cells in mixed cultures. We hypothesised that in the mix culture the signal concentration gradient will result in a lower or delayed QS response of the signal deficient population. We also hypothesised that the presence of the signal deficient population will negatively affect the QS response of the wild population. Our results demonstrated that the QS response of the wild population remains intact even in co-cultures with a high proportion (> 50%) of the signal deficient mutants. Moreover we discovered that the mutants overrespond to the QS signal at the level of gene expression and accumulation of the QS controlled public goods in the spent media. This phenotype was observed for the signal deficient strain with the mutation in signal-processing enzyme or in the gene for the signalling molecule itself but not in the mutant lacking also the signal-specific receptor. The changed response to QS signal had a dramatic negative fitness effect for the signal deficient mutant. These results therefore strongly support the hypothesis that this QS system harbours an intrinsic regulatory feedback mechanism, which is linked to the synthesis and /or processing of the QS signal. To our knowledge this is the first example of a potential ,,punishment'' mechanisms whereby the signal deficient population may pay the price for not contributing the QS signal. It also emphasizes the need for tight regulation of bacterial responses to cell-cell communication that are linked to costly secretion of public goods.

P26

***B. subtilis* social interactions: different origin, same chat**

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Bacillus subtilis communicates through the *comQXPA* quorum sensing (QS) system, which regulates genes expressed during the early stationary phase and leads to a pleiotropic response that encompasses shared production of surfactins, extracellular enzymes, and exopolymeric substances. A high polymorphism of *comQXP'* loci was found in closely related strains originating from different areas of the world. The observed polymorphism contained four communication groups (pherotypes), where strains belonging to the same pherotype exchanged information efficiently, but strains from different pherotypes failed to communicate. Here we present the morphological, metabolic and *comQXP* divergence of *B. subtilis* strains originating from two different soil environments: desert soil (Mojave desert, USA) and riverbank soil (Slovenia). Cross-activation studies using pherotype-responsive reporter strains as well as the *comQXP* loci analysis clustered Slovenian riverbank strains to three pherotypes previously identified for desert strains. However, the Ecotype Simulation (ES) algorithm using housekeeping genes (*gyrA*, *rpoB*, *dnaJ*) showed that riverbank and desert strains clustered to separate putative ecotypes, suggesting their ecological separation. Cluster analysis of the carbon utilisation profiles (Biolog) revealed that riverbank strains are metabolically highly similar, whereas desert strains are more diverse in the choice and number of carbon sources metabolised. Investigation of desert and riverbank isolates revealed different colony morphologies on nutrient poor media as well as different swarming patterns of these two groups. In conclusion, we show that despite sharing common quorum sensing languages and the ability to communicate, the strains from desert and riverbank soil differ in metabolic and morphological characteristics. This suggests that metabolism and colony morphology patterns are subject to different evolutionary forces than the traits determining the social interactions.

P27

Genetic and phenotypic diversity of *Bacillus subtilis* isolates obtained from a tomato rhizosphere

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[3]University of Maribor, Faculty of Medicine, Maribor, Slovenia

In gram-positive bacterium, *Bacillus subtilis* quorum sensing (QS) regulates various stationary phase adaptive processes, including production of surfactins, extracellular enzymes as well as induction of competence for genetic transformation. The *comQXPA* QS locus shows remarkable diversity which results in the formation of different communication groups (pherotypes). Members of one pherotype are able to communicate and thus stimulate each other, whereas strains belonging to different pherotypes cannot exchange signals. Recently, a vast diversification of pherotypes was shown to exist even on a microscale (1 cm^3). Here, for the first time we address the genetic and phenotypic diversity of *B. subtilis* isolates, obtained from a tomato rhizosphere. The strains were isolated from tomato (*Solanum lycopersicum*) roots and their phylogeney determined by sequencing *gyrA*, a housekeeping gene, and MIDI fatty acid analysis. Based on the *comQ* gene sequences and functional tests the strains from a single tomato plant were placed into three different *B. subtilis* pherotypes, which is in agreement with the diversity of QS loci previously determined for soil microscale *B.subtilis.* The rhizosphere isolates also showed a diverse morphology, haemolytic activity and the ability to develop genetic competence for transformation. Moreover, both root-growth promoting and root-growth inhibiting *B. subtilis* strains were found in the rhizosphere of a single plant. This study represents a novel approach in studying plant beneficial microbes and their interactions with plants.

P28

Molecular and cultivation based approaches to identifying bacterial interspecies interactions in Biological Soil Crusts

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Biological Soil Crusts (BSCs) are cyanobacteria-dominated assemblages that cover extensive portions of the earth's arid land surfaces. In early successional stage BSCs, such as those in the Moab desert (Utah, USA), the cyanobacterium *Microcoleus vaginatus* dominates. Through its metabolic products, this primary producer supports a limited diversity of heterotrophic bacteria that respire, ferment and assimilate its metabolites and in turn perform important functions such as nitrogen fixation. To begin to identify interactions between non-phototrophic BSC bacteria and *M. vaginatus* we performed two experiments. First, using intact BSCs, we simulated a 3-day wetting event followed by a dry down period and using 16S rRNA iTag analyses (Figure 1), accounting for compositional data we derived correlation networks to reflect the possible relationships between *M. vaginatus* abundance (rDNA) and activity (rRNA) with that of other community members. We also designed cultivation strategies to isolate a wide spectrum of heterotrophic bacteria from these BSCs in order to examine their physiological and biochemical properties that may underlie their interactions with *M. vaginatus*. Results showed that most relationships with *M. vaginatus* were positive, and particularly strong for bacterial families within the phyla Bacteroidetes and Actinobacteria (Figure 2). Conversely, a number of families within the Proteobacteria had negative correlations with *M. vaginatus* over the diel cycle. To date we have recovered over 400 bacterial isolates from BSC samples pre-incubated under dark or light conditions using media with varying carbon concentrations and antioxidants combined with extended (up to 5 months) incubations periods. These isolates were distributed over 30 different genera across 4 phyla (Actinobacteria, Proteobacteria, Bacteroidetes and Firmicutes) (Table 1). Comparisons between the identities of the isolates, metagenomic and metatranscriptomic data demonstrated recovery of more than 9% of the bacterial phylogenetic diversity by isolation - challenging the assumption that less than 0.1% of the bacterial communities in BSCs and other soils can be cultured. The isolates identified in this study represent targets for analysis of cooperative and competitive metabolic interactions that contribute to carbon flow within BSCs.

Figure 1

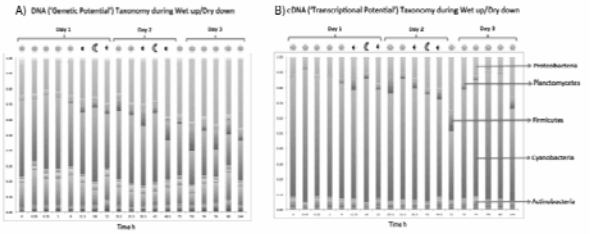

Figure Legend – Phylogenetic diversity during a wet-up dry-down event even and the network interactions of *Microcoleus vaginatus* based on the 'Genetic Potential' (DNA) and 'Transcriptional Potential' (cDNA) of the Biological Soil Crust community. (A) Genetic Potential taxonomy during Wet-up/Dry-down. (B) Transcriptional Potential taxonomy during Wet-up/Dry-down.

Figure 2

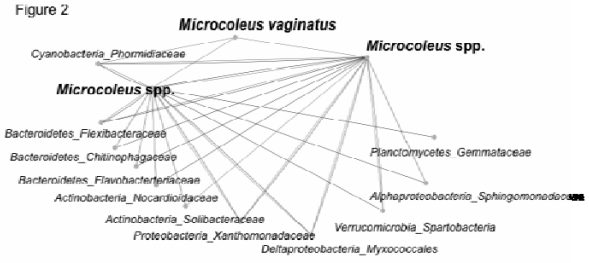

Figure Legend – Interaction Network of *Microcoloeus vaginatus* and other species in the community based in their Genetic Potential (blue) and Transcriptional Potential (pink) (Correlation >=0.6; p<=0.1).

P29

Methanotrophs and methanogens in the rice endophyte microbiome

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Metagenome analysis of root endophytes colonizing rice grown in the Philippines indicated functional characteristics of endophytes such as the presence of methane oxidizers, which are known to be present in rice paddy soils, but have not been reported before as endophytes. To reveal, whether findings on endophytes obtained from one rice cultivar sampled in one field in the Philippines have relevance on a broader scale, we sampled field-grown rice plants of several Italian and local rice cultivars grown in Macedonian rice fields. DNA and RNA of roots and stems of

plants at the ripening stage were isolated and subjected to pyrosequencing of 16S rRNA genes as well as detailed analysis on the diversity and expression of functional genes involved in methane oxidation and methanogenesis. We found highly distinct microbial communities in roots and stems showing very little overlap between the microbiota in these tissues. Roots were colonized by several taxa, which were not encountered in the stems such as Fe-oxidizing bacteria, whereas stems were colonized by *Proteobacteria*, which are known to colonize plants endophytically (e.g. *Burkholderia*, *Pseudomonas*). Methanogens were present in high abundance in roots and to a minor percentage in stems, however, methane synthesis genes (*mcrA*) were expressed in both tissues. Roots and stems hosted diverse methane oxidizing bacteria, which to a great extent expressed methane monooxygenase genes (*pmoA*). Our results indicate that the soil environment (in this case paddy soil) shapes greatly the root-associated microflora, however, above-ground tissues seem to be determined by different factors. The aspect of C1-cycling endophytes is novel and their potential relevance will be discussed.

P30
Biomass hydrolyzing enzymes identified by functional screening of a metagenomic library from algal biofilms

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Biomass hydrolyzing enzymes are increasingly searched for the production of biofuels and renewable chemical compounds using biomass. Microorganisms living on algae are an interesting reservoir of biomass hydrolyzing enzymes, as they are in constant interaction with algal biomass. Therefore, we are interested in looking for some of those enzymes synthesized by the microflora living on the surface of the brown algae *Ascophyllum nodosum*. Algae samples were collected in the winter 2012 and a microbial DNA extraction method was developed. The whole extracted microbial genomes of the microorganisms living on the algae were restricted, inserted in a cloning vector and ligated products were used for transformation of cultivable *Escherichia coli* host cells. This metagenomic library was then screened for diverse enzymatic activities (lipolytic enzymes, cellulases, beta-glucosidases, alpha-amylases, arabinanases, xylanases and proteases) on agar plates with specific substrates. Five putative lipolytic enzymes, one cellulase and one beta-glucosidase were identified. Sequence analysis revealed low (<50%) sequence identities with known enzymes sequences, meaning new enzymes from unknown genomes have been discovered. To our knowledge this is the first functional screening that was realized with a metagenomic library from algal biofilms and this is the first cellulase identified by marine metagenomics. A second library has been constructed from algae sample from summer 2012 and is currently being screened. New enzymatic tests are being developed for the identification of enzymes degrading specific algal polysaccharides like agarases, carrageenases, alginate lyases, laminarinases,... Those very specific enzymes aren't well known yet, and our metagenomic approach will probably help us to identify new families and structures of those algal biomass hydrolyzing enzymes.

P31
Sheep rumen microbiome sequencing using Ion Torrent (PGM) platform

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The microorganisms inhabiting the digestive tracts of ruminants have a profound influence on the animal development and functioning. The rumen harbors complex microbial communities dominated by bacteria, which participate in an efficient process to digest plant cell wall materials. For this reason, the rumen microbiome represents an untapped source of hydrolytic enzymes with potential application for fuel production from lignocellulosic biomass. We used the Ion Torrent (PGM) platform to access the rumen microbiome of four animals of Santa Inês breed under a base diet. In order to describe the structure of the microbial community in the sheep rumen and explore its potential as a source of biomass-degrading genes, we used two approaches, 16S ribosomal RNA Ion Tags sequencing and shotgun metagenomic sequencing. Furthermore, we measured rumen environmental parameters related to each animal, including pH, organic matter degradability (OMD), degradability of neutral detergent fiber (DNDF), total gas production (GP) and methane emissions (CH4) in order to search for correlations between these parameters and bacterial groups. In terms of microbial community structure, we found Bacteroidetes as the dominant phylum in sheep rumen microbiome, followed by Proteobacteria, Firmicutes and Actinobacteria. Some taxa were correlated with the environmental parameters, like the Corynebacteriaceae family, which was positively correlated with OMD and DNDF, and the Streptomycetaceae family, negatively correlated with GP and CH4. Some known glycoside hydrolases were identified, such as endo-1,4-beta-glucanases, beta-D-glucoside glucohydrolases, endo-1,4-beta-xylanases and others were designated as putative ones. These findings show ecological interactions among microbial groups and important rumen functions, as well as the potential of the sheep rumen for the discovery of new cellulolytic enzymes. Financial support FAPESP proc. n. 2012/03848-8.

P32
Rapid and selective colonization of fungal hyphae by antifungal bacteria in the rhizosphere

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Introduction & Question: Recent studies show that fungi can have a major contribution in the conversion of root-exudates (Hannula et al. 2012) Hence, a part of the nutrients available in the rhizosphere directly enters the fungal rather than the bacterial channel. Part of the rhizosphere bacteria may therefore be secondary consumers, i.e. growing on fungal hyphae or fungal exudates rather than on root exudates. This may change our view on interactions between bacteria and fungi in the rhizosphere. Here, we present data on the composition of bacteria that adhere

to hyphae of common rhizosphere inhabiting fungi, namely *Trichoderma harzianum* (saprotrophic ascomycete) and *Mucor hiemalis* (saprotrophic zygomycete).

Methods: We confronted the two fungi with rhizosphere bacteria extracted from two plant species (*Carex arenaria* and *Festuca rubra*) in a two compartment petri dish systems where fungal hyphae were the only carbon source available. After 24 hours of incubation, adhering bacteria and community DNA were isolated. The DNA was further analyzed by pyrosequencing (16SrDNA).Results were confirmed by microbiological assays (chemotaxis, adherence to hyphae, consumption of fungal biomass).

Results: Our main findings are that 1. A broad range of rhizosphere bacteria is able to feed on fungal exudates or the fungus itself. 2. Different fungal hosts associate with different bacterial colonizers. 3. Bacterial attachment to the fungal host happens quickly, within 24 hours.

Conclusion: These results indicate that next to plant roots, fungi may be an important carbon source for a part of the rhizosphere bacterial community.

P33

Meta-transcriptomics of the rhizosphere microbiome – the quest for bacterial genera and traits involved in natural plant protection

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In natural disease suppressive soils, susceptible plants are protected against fungal infections in spite of the presence of a virulent pathogen. Disease suppressiveness is, in many cases, microbial in origin. For various fungal pathogens, suppressiveness develops in the field after several years of high disease incidence. Hence, the fungal pathogen appears to be required for the activation of specific groups of antagonistic microorganisms. Recently, a PhyloChip-based metagenomic analysis of a soil suppressive to *Rhizoctonia* damping-off disease of sugar beet uncovered the genetic diversity and dynamics of bacterial populations in this environment (Mendes R., Kruijt M. *et al.*, Science 2011). To get further insight into the consortia of active microorganisms and functional traits expressed during interaction with the fungal pathogen, total RNA obtained from the rhizosphere of sugarbeet seedlings grown in the suppressive soil in the presence or absence of *Rhizoctonia solani* was sequenced. Analysis of the annotated bacterial rRNA tags showed that various bacterial families, mostly belonging to β- and α-Proteobacteria, Actinomycetales and Sphingobacteria, were significantly over-represented in the presence of *R. solani*. Analysis of their functional profile pointed at specific functions that were relatively more expressed by these families in the presence of the pathogen, especially functions involved in stress perception and response. The bacterial dynamics and functions highlighted by the metatranscriptome analysis were integrated in a model to try to explain how the fungal invasion of the plant rhizosphere impacts on the bacterial community and shapes an effective microbiome. Various elements of this sequence-based hypothetical model are now being tested experimentally to validate their relative importance in natural disease suppresiveness of soils.

P34

***Allolobophora hrabei* and its effects on soil microorganisms in steppe ecosystems**

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Ecology of the earthworm *Allolobophora hrabei* is untypical due to its strong preference for steppe and dry grassland soils and extremely high casting and burrowing activity. There is lack of information about the impact of this giant endemic earthworm on the soil environment.

In our research, we aimed to map microbial changes in soil inhabited by *A. hrabei.* Three experimental sites located in South Moravia (Czech Republic) with active populations of A. hrabei have been monitored since spring 2012. Viable biomass, structure of aerobic and anaerobic microbial community has been evaluated by the extended phospholipid fatty acids analysis (PLFA). Cultivation and MIS Sherlock analyses (MIDI Inc., USA) have been used for bacterial counts, growth strategy, and dominant species determination in soil, drilosphere, and worm casts.

The pilot monitoring indicated the worm casts as the hot spots that have significantly differed from surrounding soil and drilosphere. The microbiome of casts was typical by PLFA composition, significant increase of microbial biomass, enriched by aerobes, dominated with cultivable Actinobacteria and Firmicutes, and favouring slow growth strategy.

Our investigation (i) contributes to our knowledge on microbial diversity in specific habitats formed by this endemic earthworm, (ii) helps to understand soil functioning in fragmented Central European steppe ecosystems, and (iii) may improve maintaining the biodiversity in such endangered ecosystems.

P35

How do the polyphenol oxidases of *Streptomyces* affect plant growth promotion?

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Two polyphenol oxidases (PPOs) produced by *Streptomyces* exhibit opposite effects on plant growth promotion (PGP), *i.e.*, extracellular MelC2 enhances plant growth, whereas intracellular MelD2 diminishes it. To investigate the physiological pathways of plants involved in these effects, we performed microarray analysis of *Arabidopsis thaliana* Col-0 seedlings inoculated with different *melC* and *melD* strains of *Streptomyces*. The results showed that the presence of *melC* and the absence of *melD* in *Streptomyces* similarly induced or repressed certain circadian clock related genes, TOC1, CCA1, COR27, COL9 and PRR3 in *Arabidopsis*. To study the role of circadian clock in the observed PGP effects, four circadian clock mutants (*toc1*, *cca1*, and *cor27, lwd1wld2*) of *Arabidopsis* were tested. No effect of PGP the PPOs

was observed in the four mutants. Moreover, changing the growth condition of *Arabidopsis* from long daytime (16-h light:8-h dark) to short daytime (8-h light:16-h dark) also eliminated the PGP effects. To understand the involvement of plant defence system in the PGP effects, *Arabidopsis* mutants in systemic acquired resistance (SAR) and induced systemic resistance (ISR) were tested. The involvement of SAR was ruled out by tests using the *npr1* mutant. In contrast, tests using *ein2-1* (ethylene insensitive) and *jar1-1* (methyl jasmonate insensitive) mutants revealed the involvement of ethylene pathway was involved in the effect of MelD2, and jasmonic acid pathway in the effect of MelC2. This indicated that the two PPOs act through separate pathways in ISR. Furthermore, both $MelC^{+}$ and $MelD^{-}$ strains produced higher indole-3-acetic acid (IAA) than the wild type. Knockout of IAA biosynthesis eliminated the PGP even in the presence of MelC. These results suggest the involvement of IAA also.

P36

Opposite effects of two polyphenol oxidases of *Streptomyces* on plant growth promotion

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Filamentous bacteria *Streptomyces* are among the most abundant bacteria in soil. While a small fraction of *Streptomyces* is plant pathogens, many associate with healthy plants with beneficial effects, *e.g.*, promotion of plant growth (PGP) and of symbiosis between plant roots and other microbes. In rhizosphere, plants secrete wide range of phenolics which are substrates for two homologous polyphenol oxidases (PPOs) produced *Streptomyces* — a universally present intracellular MelD2 and a sporadically present extracellular MelC2. Supposedly, in side the mycelia, MelD2 decreases the toxicity by replacing the spontaneous ROS-generating oxidation of phenolics, whereas the extracellular MelC2 increases the sensitivity by converting the phenolics into more permeable hydrophobic quinones. We discovered that two PPOs also exhibit opposite effects on PGP, *i.e.*, MelC2 increases PGP, whereas MelD2 reduces it. The opposite effects were observed in eight of eleven plants tested in the soil, and also in *Arabidopsis* on agar. The results from agar ruled out the involvement of other microbes and soil materials (which may be processed by extracellular enzymes of *Streptomyces*). The latter was supported by the lack of effect of mutations in the major protein secretion pathway (twin-arginine transport) of *Streptomyces* on the PGP. In seed adhesion assay of corn, the presence of *melC* or the absence of *melD* reduces the adhesion of the mycelia to about 10% of the wild type. The investigation of the pathways involved in the observed PGP effects is presented in the accompanying poster.

P37

***Mesorhisobium medetiranium* and *Mesorhisobium tianshanense* - simbionts of sainfoin**

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The genus *Onobrychis*, the sainfoin, is perennial herbs of the legume family (Fabaceae), which is represented by 80 species. They grow in Europe, Mediterranean, Western Asia and the USA. There are a lot of specific and ecological varieties of the sainfoin in natural flora. The main cultivated species are *O. Viciafolia*, *O. Antasiatica*, *O. Arenaria*.

It is known that the symbiotic bacteria species *Phyllobacterium, Rhizobium*, and *Mesorhizobium* can form the nodules on legume roots genus *Onobrychis* and can thus fix atmospheric nitrogen. However, they haven't been examined in details. That is why the previous investigations of taxonomy of sainfoin's nodule bacteria did not give the whole notion about the simbiont's biodiversity. So the investigation of phylogeny of sainfoin's simbionts by complex approach targeted to investigation of housekeeping and symbiotic parts of a genome is actual and perspective task.

In prairie and foothill regions of Crimea for the first time 10 isolates of symbiotic bacteria from sainfoin's nodules were collected. These nodule bacteria were analyzed using fingerprinting saAFLP with rare-cutting restriction enzymes *XmaJI* and *XbaI*, and also by analysis of nucleotide sequences of internally transcribed spacer region between the *16S* and *23S* rRNA genes (ITS), *16S* rRNA, *gyrB*, and symbiotic *nodC* and *nifH* genes.

Based on obtained results, it was shown all investigated isolates belonged to species *M. medetiranium* and *M. tianshanense*. saAFLP analysis allowed to investigate an inter-specific biodiversity of the natural populations of sainfoin's symbionts in Crimea. Firstly almost complete sequences of *nodC* and *nifH* genes were determined. All sequences were deposited into GenBank NCBI and were also loaded on our website:http://hin-project.com/.

Studies were held within the cooperation agreement of A.N. Bach Institute of Biochemistry of Russian Academy of Sciences and Department of Microbiology, Institute of Agriculture of Crimea of National Academy of Agriculture Sciences of Ukraine. The work was made with financial support of «Greenwide» and of the «Support of young scientists» programme of Russian Academy of Sciences.

P38

Distinct bacterial communities inhabit the digestive tract of *Escherichia coli* O157:H7 super-shedding cattle

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Introduction: Cattle are the primary reservoir for *Escherichia coli* O157:H7 but highly variable shedding patterns of this food borne pathogen are often observed between different host animals.

Super-shedders (individuals shedding >10^4 *E. coli* O157:H7 CFU/g of feces) are logical targets for mitigation strategies aimed at reducing the incidence and spread of this pathogen. Recently, the composition of the fecal bacterial communities were found to be significantly different between super-shedder and *E. coli* O157:H7 negative cattle suggesting an important role of the microbiota in the super-shedding condition.

Objective: To compare bacterial communities along the digestive tract of cattle shedding or negative for *E. coli* O157:H7.

Materials and Methods: This study used pyrosequencing of 16S rRNA genes to examine the intestinal bacterial communities of 6 cattle (3 super-shedder and 3 *E. coli* O157:H7 negative). A total of ≈ 350,000 pyrotags were produced from DNA extracted from digesta samples from the duodenum, jejunum (proximal, mid and distal), cecum, colon (descending and spiral) and rectal-anal junction (RAJ), as well as scrapped tissue from the RAJ to evaluate adherent populations in this area.

Results: The bacterial communities for digesta samples taken from the small intestine (duodenum to jejunum) were not significantly different for super-shedding and *E. coli* O157:H7 negative cattle. In contrast, there were significant differences between the two study groups in their large intestine (*i.e.*, cecum to the terminal RAJ) bacterial communities. Super-shedding animals had significantly more diverse communities than negative animals.

Conclusion: These results support the hypothesis that the super-shedding condition is the result of an intestinal dysbiosis in cattle.

P39
ECF sigma factors – how endophytes sense the plant

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Endophytes are fungi or bacteria that spend at least one phase of their life span inside a plant without causing disease symptoms in their host. Today, it is widely accepted that bacterial endophytes actively grow within and interact with their host plant. However the mechanisms of plant-bacteria communication are still poorly understood. How for example do bacteria recognize the plant environment? Do they feel plant stress and how do bacteria respond to plant stimuli?

One way how bacteria sense and react to the extracellular environment is the so-called cell surface signalling (CSS) employing alternative sigma factors. This signal transduction system consists of an outer membrane receptor, an inner-membrane bound sigma factor regulator (anti-sigma factor) and bound to that an extracytoplasmatic function (ECF) Group IV sigma factor. Upon signal recognition the ECF sigma factor is released and activates expression of its target genes. Recently, the genome of the plant growth promoting endophyte *B. phytofirmans* (PsJN) has been fully sequenced (Weilharter et al., 2011) and 18 CDS have been annotated as putative sigma factors.

The main aims of the present study are (i) to identify ECF sigma factors that are involved in the interaction between strain PsJN and its host plants (ii) to quantitatively assess ECF sigma factor activation in response to plant stimuli in different stress conditions (iii) to identify ECF sigma factor genes that are involved in plant colonization and plant growth promotion activity in *B. phytofirmans* (PsJN) and (iv) to identify genes that are regulated by given ECF sigma factors in *B. phytofirmans* (PsJN).

Specific and efficient primers and probes were designed for each ECF sigma factor gene and Taqman-quantitative PCR is used to identify ECF sigma factors that are expressed in response to plant signals. These ECF sigma factor genes will be disrupted and mutants tested for their ability to establish populations in and to promote growth of micropropagated potato plantlets. Overexpression of selected ECF genes and subsequent transcriptome comparison to a not induced control will identify genes that are regulated by a given ECF sigma factor in response to plant stimuli.

P40
The host microbiome and plant health – seed colonizing microbes from disease suppressive vermicompost alter zoospore chemotaxis, encystment and germination of *Pythium aphanidermatum*

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Many oomycete plant pathogens rely on the production of motile infective propagules, zoospores, to seek out hosts in the soil environment. *Pythium* spp. are susceptible to suppression by microbially rich substrates and the zoospore stage may be particularly vulnerable. However, insight into the plant-microbe and microbe-microbe interactions responsible for this suppression is lacking. Rather than examining the suppressive substrates' entire microbial community, we focused on the host, the pathogen and the host-associated microbes in the infection court at a temporal scale relevant to infection.

We sought to explore the impact of a suppressive host microbiome on pre-infection events carried out by *Pythium aphanidermatum* zoospores; chemotaxis, encystment and germination.

In *in situ* bioassays, zoospores were forced to swim 2 cm to reach hosts which had been pre-germinated in the suppressive substrate. Zoospore arrival on the seed surface was validated with qPCR. Exudates from seeds sown in vermicompost for 8 hours then transplanted to sand for 24 hours (microbially modified seed exudates: MMSE) were collected, sterile filtered and used for *in vitro* zoospore assays. MMSE was compared to exudates from seeds sown in sand via metabolomic analyses.

For *in situ* assays, less zoospore DNA was detected on seeds pre-germinated in vermicompost and transplanted to sand prior to inoculation than seeds pre-germinated in sand. *In vitro* exposure to MMSE inhibited chemotaxis, encystment and germination of zoospores relative to these responses to exudates from seeds sown in sand. Combining control seed exudate with MMSE failed to restore suppression and extensive zoospore lysis was observed, providing evidence for the presence of a zoosporolytic compound.

Metabolomic analysis of the seed exudate identified18 putative active compounds characterized as free fatty acids or unknown compounds.

We conclude that the plant host rapidly recruits a suppressive sub-set of the total microbial community within the first 8 h of germination. This spermosphere microbial community chemically alters seed exudates in such a way that interrupts zoospore pre-infection events and ultimately protects the host from disease. Our results confirm the important role the host microbiome plays in maintaining plant health.

P41

Two-component systems signalling systems in the *Azospirillum*: from comparative genomics to functional gene analysis

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Introduction: Two component signal transduction systems (TCSs) are key mediators of signal transduction. Aside from the paradigm phosphotransfer pathway involving one histidine kinase (HK) and one response regulator (RR), more complex versions of TCS exist with multiple phosphotransfer reactions (HyHK). *Azospirillum* is a plant growth-promoting rhizobacterium (PGPR) living in the rhizosphere of many important crops. However little is known about how the bacterium senses and responds to the external signals encountered at the vicinity of roots.

Objectives: The aim of this study is to identify and characterize TCS encoding genes potentially involved in *Azospirillum*-plant adaptation.

Materials & Methods: Data including the identity, features and replicon position of all the TCS genes from three available complete *Azospirillum* genomes were obtained using the P2CS database. Numbers and architecture of azospirilla's TCS were compared with those found in 83 completely sequenced bacteria inhabiting the rhizosphere. Four mutant encoding HyHKs displaying complex domain architecture were constructed and analysis of their phenotype was undertaken.

Results: *A. lipoferum* 4B harbors 47 genes encoding HyHK, making it one of the bacterium with the largest numbers of HyHKs among plant-associated bacteria sequenced to date. Moreover, HyHKs display complex domain architecture compared to their classic HK counterparts. One of the mutant inactivated in a plasmid-encoded HyHK displays stronger oxidative stress resistance, modification of the cell morphology and enhanced aggregation properties when compared with the parental strain. Moreover, the pink pigmentation of this mutant suggests the involvement of the corresponding HyHK in carotenoid biosynthesis. Structure of the cognate RR indicates a potential link between the c-di-GMP second messenger and regulation of these multiple phenotypes.

Conclusion: The existence of a large number of HyHKs indicates that they play a significant role in multiple-step phosphorelays in *Azospirillum*, likely by fine-tuning multiple genes related with its plant-associated lifestyle.

P42

Host specificity of the plant growth-promoting cooperation between *Azospirillum* and rice

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Introduction: Host specificity is a fundamental concept in understanding evolutionary processes leading to intimate interactions between bacteria and plants. In the case of Plant Growth-Promoting Rhizobacteria (PGPR) specificity appears to be controlled either by a strain-specific bacterial adaptation to non-specific traits of the host plant or by non-specific bacterial adaptation to genotype-specific properties of the host plant (1). Thus, we hypothesize that these adaptations result in the regulation of a large number of genes, independently of their direct involvement in phytostimulation. These regulations may depend on the bacterial strain/plant genotype combination.

Objectives: This work aims at identifying genes involved in reciprocal adaptation of partners and those involved in host specificity in PGPR-plant cooperation.

Materials & methods: To evaluate transcriptomic responses of each partner during the *Azospirillum*-rice cooperation, RNA samples obtained from *Azospirillum* root-associated cells and rice roots (cultivars Nipponbare and Cigalon) were analyzed on microarrays.

Results: Transcriptomic analyses evidence the regulation of 453 genes in root-associated *Azospirillum* cells and 7384 genes in rice roots. Whereas none of the *Azospirillum* properties involved in the modification of plant hormonal balance are significantly regulated under the experimental conditions, several genes of the plant partner implicated in phytohormone signaling are induced or repressed during the interaction. The induction of plant and bacterial genes involved in ROS detoxification suggest that defense response of the host plant play a key role in *Azospirillum*-rice cooperation. In addition, many genes display expression profile that depends on the strain/cultivar combination.

Conclusion: Combination specific responses observed at the transcriptomic level are consistent with metabolomic observations previously reported for *Azospirillum*-rice cooperation (2), suggesting that evolutionary processes have led to a preferential interaction between a strain and its original host cultivar.

References

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P43

Genome and transcriptome analysis reveals mechanisms involved in beneficial plant-microbe interaction of the Stress Protecting Agent *Stenotrophomonas rhizophila* DSM14405^T

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Stenotrophomonas rhizophila DSM 14405^T promotes plant growth in a wide variety of crops under stress conditions. A significant increase in plant growth was particularly observed in salinated and arid soils. Since little is known about the mode of interaction we have sequenced the genome of *S. rhizophila* DSM 14405^T as basis for studying the molecular and physiological mechanisms underlying the root system function by using both a transcriptomic approach as well as root colonization assay with confocal laser scanning microscopy under different conditions. Ecto- and endophytic colonization of roots could be shown for all studied crops (oilseed rape, cotton, tomato and sweet pepper) resulting in positive effects on root and plant development. In response to seedling extracts, the transcriptome of *S. rhizophila* DSM 14405^T changed drastically. Several of the genes with altered expression levels are known to directly or indirectly promote plant growth, and we additionally observed that the transcription of genes important for host cell attachment, plant cell colonization, chemotaxis and motility, substrate transport and excretion through channels, amino acid metabolism and transport were also significantly influenced. Overall, the genome of *S. rhizophila* DSM 14405^T comprises genes featuring ecological fitness and intimate interaction with plants. Based on the transcriptomic analyses, plant seedling extracts specifically trigger genes involved in beneficial interactions between *S. rhizophila* and plant roots. Furthermore, competent root colonization was identified as a key factor for these beneficial effects.

P44

Warning - bacterial stink bomb! The potential of non cyanogenic *Pseudomonas* for biological control of *Phytophthora infestans*

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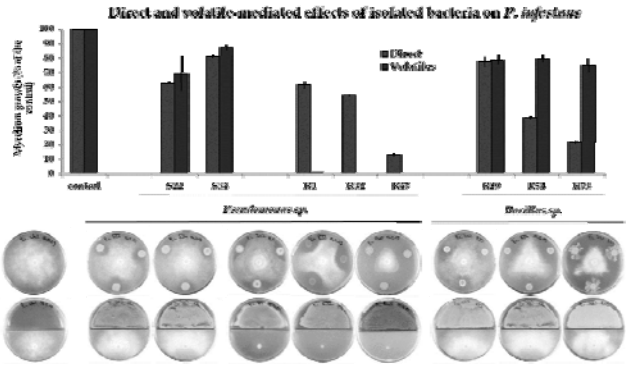
During the last decade, the importance of bacterial volatiles in cross-kingdom interactions has become clearer and clearer. In addition to promoting plant growth and root development, bacterial volatiles have been repeatedly shown to inhibit the growth of phytopathogenic fungi, although the molecules responsible for this effect are still largely unknown, with the notable exception of hydrogen cyanide. Most research performed so far has dealt with *Rhizoctonia* or *Fusarium* species, however, hardly anything is known at present about how fungi-like organisms such as oomycetes would react to bacterial volatiles. We are investigating this question with *Phytophthora infestans*, the causing agent of potato late blight. Bacteria were isolated from roots and shoots of field-grown *P. infestans*-infected potato plants and screened for volatile-mediated oomycete growth inhibition. The most active strains turned out to belong to the genus *Pseudomonas*, three of which being non cyanogenic but producing volatiles, which caused complete growth arrest in the oomycete. Further analyses will reveal i) which volatile molecules account for the observed effects, ii) which physiological changes are caused in the oomycete by exposure to bacterial volatiles and iii) how efficient the strains are in fighting *in planta*-grown *P. infestans*.

Figure legend:

Susceptibility of P. infestans *to diffusible (grey) and volatile (red) compounds from 5* Pseudomonas *and 3* Bacillus *isolates. Mycelium growth was monitored after seven days of co-cultivation. All data significantly different from the control, n=3 P<0.01*



P45

Comparative analysis of different *Burkholderia* genomes reveals the basis of fungal interactive bacterial strategies

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Genus *Burkholderia* is known for its ability to interact with eukaryotic animals, plants and fungi. Recently, two *B. terrae* strains denoted BS001 and BS110 were shown to interact with soil fungus *Lyophyllum sp.* strain Karsten, both forming biofilms around fungal hyphae and migrating along these in soil. The ability to migrate with extending hyphae was then shown to be present among *B. terrae, B. terricola, B. xenovorans* and *B. phytofirmans.*

To better understand the mechanisms behind the *Burkholderia*-fungal interactions, we obtained Illumina draft genome sequences from 7- strains, four (BS001, BS007, BS110 and BS437) related to *B. terrae* and three (BS455, J1U5 and BIFAS53) to *B. phytofirmans*. Strains J1U5 and BIFAS53, came from *Diapensia lapponica* in low-arctic fell tundra in Finland.

Estimated genome sizes were 8.2-11.5 Mb, with *B. terrae* strains having bigger genomes (11.0-11.5 Mb) than *B. phtofirmans* (8.2-10.3 Mb). The genomes possessed genetic systems encoding chemotaxis, motility, signalling and (type 3, 4 and 6) secretion systems. *B. terrae* had more genes (e.g. type 6) for these functions than *B. phytofirmans*. Furthermore, *B. phytofirmans* strains J1U5 and BIFAS53 lacked T3S-machinery, whereas only partial T3SS was detected in BS455. Genes for biofilm formation were differentially present in *B. terrae* strains. BS007, BS110 and BS437 possessed both *pga* (a-c) and *PEL*(a-g) gene systems, while BS001 had only *pga*. *B. phytofirmans* BS455 had only the *PEL* system, whereas the *B. phytofirmans* arctic strains BIFAS53 and J1U5 had none. Correlation between secretion systems and migration will be explained in meeting. Large sets of carbohydrate acquisition and metabolism genes *e.g.* for glycerol, were found in all genomes. On the other hand, *B. phytofirmans* BS455 contains high number (52) of genes for iron acquisition and

metabolism while the minimum (13) was in *B. terrae* BS007 and BS437.

Strain BS001 did not reveal any gene for invasion and virulence while all others contained 13-14 such genes. In contrast, all *B. phytofirmans* strains contained genes potentially relevant with their interactions with plants e.g. *AcdS*, enconding ACC deaminase, as well as genes for synthesis and metabolism of other plant hormones. Interestingly, some, like *AcdS* and genes for auxin biosynthesis were also present in the *B. terrae* strains except BS001.

P46

The characteristics and putative ecological role of a new IncP-1β plasmid, pHB44, found in *Variovorax paradoxus* like strain HB44 isolated from the mycosphere of *Laccaria proxima*

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Bacterial-fungal interactions are common in a wide variety of habitats, especially in soil. The organisms may compete, cooperate or be neutral with each other, and so different interactions may occur in soil between bacteria and fungi. For the bacteria, the mycosphere is important and bacteria may have evolved in a way that they can benefit from soil fungi. The putative role of plasmids in the bacterial fitness gains in the mycosphere has been underexplored. We provide evidence for the occurrence of IncP1**β** plasmids in a group of *Variovorax paradoxus* related bacteria isolated from the mycospheres of ectomycorrhizal fungi like *Laccaria proxima* in the field. Specifically, we found plasmids in two out of 15 *V. paradoxus* related strains at two occasions. The 60-kb plasmid of strain HB44, denoted pHB44, was selected for further analyses. It was shown to be capable of transferring an indicator plasmid, pMOL187, to an *Escherichia coli* recipient strain and was also self-transferable. It thus contained a fully functional transfer system. Plasmid pHB44 was devoid of antibiotic or heavy metal resistance, or biodegradative genes, and no phenotype other than gene mobilizing capacity could be attributed to it. PCR-based analysis based on assumptions as to hot-spot insertion sites revealed the presence of a region of about 14 kb, denoted hot spot 2, where accessory genes occurred. Analysis of the partial sequence of pHB44, obtained by pyrosequencing, showed the presence of the canonical IncP1**β** plasmid backbone, in which batteries of genes for replication and stable maintenance on the one hand, and horizontal transfer on the other hand, were clearly present. Evidence was found for the contention that the plasmid backbone had a mosaic structure, consisting of one part identical to plasmid R751, whereas another part was akin to plasmid pAMMD1. Additional exogenous isolations have confirmed the prevalence of mobilizer plasmids of the IncP1beta type across four different mycospheres. We are currently analyzing such plasmids as to type, diversity and putative role.

P47

The ecology of microbial communities associated with the traps of aquatic carnivorous *Utricularia* species

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Utricularia are the most versatile and cosmopolitan among carnivorous plants. There has recently been renewed scientific interest in this rootless genus, focused on plant-microbe interactions in *Utricularia* traps, which are known to harbour diverse microbial communities. As the decisive role of carnivory in *Utricularia* nutrition has been debated, we have focused on the possible role of trap microorganisms in enhancing plant nutrient aquisition, especially in nutrient poor environments, where prey capture rates are low. Bacteria were found to constitute approximately 50% of total viable microbial biomass as determined by PLFA analysis. Preliminary results revealed the presence of various nitrogen fixing bacterial species; however, ^{15}N labeling has shown that fixed nitrogen is not a significant nutrient source for the plants. Polyphosphate granules were found to be abundant in the trap microbial community, with polyphosphate constituting up to 60% of total particulate phosphorus content of the trap fluid. Viable nannoflagellate and ciliate grazers have been identified inside the traps, and sigficant grazing on bacterial cells has been confirmed. We argue that the presence of a fully-functional microbial food web within *Utricularia* traps, capable of regenerating nutrients from complex organic sources such as humic substances, may present an important nutritional benefit for the plants, which are commonly associated with nutrient-limited environments.

P48

The diversity and biogeography of bacterial communities associated with pioneer plant species in the Arctic

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Arctic and subarctic ecosystems cover 22% of the earth's terrestrial surface, but remain poorly characterized. For example, the factors affecting vegetation dynamics and colonization of unvegetated areas and, inversely, impact of vegetation on soil microbial communities, are not well understood. We used 16S rRNA gene targeted pyrosequencing (454) to compare the community composition of bacteria associated with two arctic pioneer plant species, *Oxyria digyna* and *Saxifraga oppositifolia.* To test the effect of plant species, site and climate on community composition, we analyzed 58 samples representing rhizosphere and endosphere of the two plant species and bulk soil from low and high Arctic (69ºN and 78ºN, respectively).

The endosphere bacterial communities were clearly less diverse than soil communities in all sampling sites. In contrast, there were no significant differences in community diversity or richness between sites (or climates). Analysis of bacterial phyla distribution revealed clearly higher abundance of Firmicutes and Proteobacteria in endosphere samples as compared to rhizosphere or bulk soils in all sites. In contrast, Acidobacteria, Actinobacteria and Nitrospira were relatively more abundant in soil samples.

Further, the actinobacterial classes in soils and in endopsheres were clearly divergent. Interestingly, Actinobacteria had higher relative abundance as well as higher diversity in high Arctic than in low Arctic sites, regardless of sample type (soil of endopshere).

Correspondence analysis on bacterial phyla level revealed that community composition in endosphere as well as in rhizosphere samples was strongly dependent on host plant species rather than site (or climate). However, class level analysis revealed a strong effect of sampling site on community composition. *S. oppositifolia* endosphere communities were rich in Δ-Proteobacteria and Firmicutes. Interestingly, most of the Firmicutes in *S. oppositifolia* endosphere samples represented *Clostridia* sp. Bacterial subclass and family level analyses revealed further plant host specific as well as biogeographical trends.

P49

Behavior *in vitro* of rumen ecosystem using different grazing agroecosystems

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The rumen plays specific roles in the digestive system of ruminants and it is considered an ecosystem. This ecosystem hosts a microbial community that is characterized by high population density, wide diversity and complexity of interactions. The activity of these organisms enables food degradation and the formation of end products that may be beneficial or detrimental to the host and to the environment. Several factors influence the ruminal ecosystem functioning, one of them is the diet that is given to animals. The aim of this work was evaluate, *in vitro*, the influence on the ruminal microbial ecosystem active in different grazing agro-ecosystems.

Methods: The *in vitro* gas production technique was used to predict the rumen fermentation and microbial community activities. The fermentation process was carried out during 24 hours. The ruminal fluid donor animals were 06 males Santa Ines sheep. Five experimental diets were evaluated corresponding to grazing agro-ecosystems: 1) Legume mixture associated with grasses, 2) Biomass Bank, 3) Degraded grass area, 4) Integrated forest-livestock system (*Leucaena* - guinea grass), 5) Star grass *(Rhinchospora nervosa)* monoculture. It were determined the gas and methane production, the concentration of short chain fatty acids (SCFA), N-NH_3 and the quantification of the populations of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and total methanogens were determined by real time-PCR.

Results: The results showed that all grazing agro-ecosystems favored similar abundances of rumen populations of methanogens, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*. In all cases methanogens were mostly represented than other rumen microbial populations . Concentrations of N-NH_3 did not differ among the experimental diets and was higher than that required to achieve an optimum rate of fiber digestion in the ruminal ecosystem. Ruminal concentrations of SCFA (acetic, propionic and butyric acid) and methane production were also similar among the studied grazing agro-ecosystems. The fermentation pattern was acetic, characteristic of fibrous foods. The gas production was higher with the use of legumes mixture associated with grasses and the biomass bank.

Conclusions: It was concluded that grazing agro-ecosystems did not adversely affect the ruminal ecosystem as exhibited similar growth and functional patterns of the microbial populations and fermentation products derived from their activities.

P50

Large scale monitoring of *Mycobacterium bovis* prevalence in the Eurasian badger (*Meles meles)* using a faecal based, non-invasive QPCR method

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Mycobacterium bovis is the causative organism of tuberculosis (TB) in a number of mammalian hosts including cattle and badgers. In the UK a strict test and slaughter program is used in an attempt to reduce the incidence and spread of TB within and between cattle herds. As a result, in 2011 over 25,000 cattle were slaughtered and the cumulative cost of compensation, testing and research exceeded £90 million. It is predicted that bovine TB will cost the UK economy in excess of £1 billion over the next 10 years if no further interventions are implemented.

The Eurasian badger (*Meles meles*) is thought to form a self-sustaining reservoir of *M. bovis* infection. Although classically thought of as a respiratory disease, systemic infection is common within badgers and shedding of *M. bovis* into the environment occurs through multiple routes, for example sputum, urine and faeces. Environmental reservoirs of *M. bovis,* e.g. contaminated faeces, are a potential transmission route between badgers and cattle with some faeces containing up to 10^8 cells per gram. Furthermore, *M.* bovis can persist at detectable levels within badger faeces for a significant period of time (one year). In order to further the understanding of *M. bovis* transmission between reservoir populations and cattle, a greater understanding of the general ecology of *M. bovis* within badger populations is required.

A non-invasive QPCR method was used to detect and quantify *M. bovis* in badger faeces to monitor a highly studied wild badger population known to have a high TB incident rate. Over 2000 badger faecal samples were obtained from 12 social groups over a year long period. *M. bovis* presence and load quantification was determined using a QPCR method targeting the RD4 deletion region unique to the *M. bovis* genome. These results were matched with trapping and traditional testing regimes, carried out concurrently, to determine the minimum sampling intensity needed to accurately describe the potential infectivity of *M. bovis* in this population. This information is essential when aiming to design and monitor strategies for TB control in badgers. The test was also used to determine efficacy of an orally delivered vaccine in badgers and preliminary analysis indicated variation in shedding between animals under different vaccine regimes.

P51

Diversity of endophytes fungi associated to two different medicinal plants

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Medicinal plants are those that are capable of produce secondary metabolites. Brazil is the country that has the major diversity of plants involved in a worldwide production of synthetic drugs such as procaine, chloroquine and tropicamide, used to control human diseases. Nevertheless, in complex ecosystems plants may be associated with others microorganisms in a symbiotic relationship by means of exchange of benefits, where plants supply the fungi with some carbon compounds and fungi help plants against pests. In the same way, some endophytes fungi may act as partners in a synergistic relationship by producing the same secondary metabolites as plant, increasing its production. In this context, the present work aimed to describe the fungal diversity endophyticaly associated to two Brazilian medicinal plants: *Cymbopogon citratus* and *Melissa officinallis* cultivated in two different substrates along one year cultivation period. The isolated fungi were characterized using microculture techniques, and then identificated by both, morphological and sequencing the *ITS* (*Internal transcribed spacer*) region, techniques. The results showed that there were no significantly difference on the isolation frequency along a cultivation period but qualitatively differences, were observed over the plant genotype as *Xylaria sp., Nigrospora sp.* and *Dreschlera sp.* predominantly associated with *M. officinalis and Geotrichum sp., Ampelomyces sp.,* associated with *C. citratus.* Hence the use of classical and molecular techniques in conjunction, have been applied to shed light in the high fungal diversity and "fine-tune" the research over this association (Plant-fungi), in order to change the magnitude of the secondary metabolite production.

P52

Comparison of two assays for the screening of phytogenics against *Lawsonia intracellularis*

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Introduction: *Lawsonia intracellularis* (LI) are Gram-negative intracellular bacteria that are of economic importance in pig husbandry as the cause of proliferative enteropathy (ileitis). While the acute form of ileitis can cause sudden mortality, the chronic and subclinical forms can drastically impair growth performance. Phytogenics are plant-derived materials used as feed additives in animal production with the aim of exerting a beneficial influence on health and growth.

LI do not proliferate in a cell-free medium. This means that conventional bacterial inhibition assays like agar plate or broth micro dilution assays cannot be used to screen samples for activity against LI.

Objectives: The objective was to develop an assay to screen phytogenic samples for their activity against LI. Two methods were compared: A viability assay, evaluated by flow cytometry, and an assay involving host cells, evaluated by a microplate reader. Several samples (phytogenics with known antibacterial activity, and antibiotics) were tested with both methods.

Materials & methods: LI were reconstituted from a live vaccine (Boehringer Ingelheim, Germany), filtered and washed to remove debris.

For the viability assay, LI were incubated with phytogenic samples, stained with fluorescent dyes and analyzed with a flow cytometer (ACCURI C6). Viable and damaged bacteria were distinguished via fluorescence signals.

For the host cell assay, LI were co-cultured with McCoy mouse fibroblast cells in 96 well plates in the presence of samples. After 5 d the cells were fixed and intracellular bacteria were stained with a primary anti-LI and a secondary fluorescein-conjugated antibody. The fluorescence of intracellular bacteria was measured with a microplate reader.

In both assays untreated and sample-treated LI were compared to assess the relative inhibition by phytogenics.

Results: In both assays, several phytogenic samples showed antibacterial activity. Among the used antibiotics tylosin tartrate was able to inhibit LI in the host cell assay, but showed no activity in the viability assay.

Conclusion: *In vitro* screening assays like those presented in this study shall be the first step for the development of a phytogenic feed additive for LI control.

P53

Tackling the specificity of the marine sponge microbiome: a biogeographical approach

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Geographical isolation plays a crucial role in driving microbial evolution and community structure in nature. In this context, the study of microbial symbionts in animal hosts of restricted mobility is of particular interest given their contribution to host fitness and survival. Here, we inspect the extent to which the shape of the sponge-associated microbiome is driven by the host organism and its biogeographical background. To this end, specimens of the sponge genera *Ircinia, Sarcotragus* (Dictyoceratida, Irciniidae) and *Spongia* (Dictyoceratida, Spongiidae) were sampled at distinct biogeographical settings in the North Atlantic: the Algarve coast, the Madeira Island, and the Azores archipelago. Bacterial community profiling in marine sponges was performed by 454 pyrosequencing of 16S rRNA genes amplified from metagenomic DNA samples. Analysis of 133959 chimera-free sequences was performed using the QIIME pipeline. *Ircinia* spp. possessed a more variable microbiota than *Sarcotragus* spp. . Surprisingly, bacterial communities from the former - and phylogenetically closer - species were less similar to one another than *Spongia* spp. and *Sarcotragus* spp. communities The predominant taxonomic groups

in *Sarcotragus* and *Spongia* were Acidobacteria and Actinobacteria (*c.* 20% relative abundance), followed by Proteobacteria, Poribacteria, PAUC34f, Bacteroidetes and Chloroflexi (*c.* 10% relative abundance each group). The aforementioned abundances remained similar in these sponges regardless of the sampling locality. Conversely, phylum-level abundances shifted across localities in *Ircinia* spp. In these hosts, the Chloroflexi displayed dominance at the Madeira site (31%) but was found to be a minority taxon at the Algarve (7%) and Azores sites (3%), where Proteobacteria, Acidobacteria and Actinobacteria were dominant groups at varying degrees. *Ircinia* spp., the most variable host regarding symbiont community composition, maintained a common pool of symbionts (52 shared bacterial phylotypes in 173 detected) across all sample sites examined. Overall, our results suggest that the sponge host plays a pivotal role in shaping the structure of its associated microbiome across biogeographical gradients.

P54

Influence of *Bacillus amyloliquefaciens* FZB42 on the disease severity of bottom rot and the rhizosphere microbial community of field grown lettuce (*Lactuca sativa*)

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The soil-borne pathogen *Rhizoctonia solani* is responsible for crop losses on a wide range of important crops worldwide. The lack of effective control strategies and the increasing demand for organically grown food has stimulated research on biological control. The aim of the present study was to evaluate the rhizosphere competence of the commercially available inoculant *Bacillus amyloliquefaciens* FZB42 on lettuce cultivated in a field infested with *R. solani* and to determine its impact on indigenous rhizosphere bacterial community. Results demonstrated that FZB42 is able to effectively colonize the rhizosphere of lettuce and to reduce the severity of bottom rot caused by *R. solani* after treatment of young plants with FZB42 before and after planting. The 16S rRNA gene based fingerprinting method terminal restriction fragment length polymorphism (T-RFLP) showed that the treatment with FZB42 did not have a major impact on the indigenous rhizosphere bacterial community. However, the bacterial community showed a clear temporal shift. The results also indicated that the pathogen *R. solani* AG1-IB affects the rhizosphere microbial community at higher pathogen pressure. Thus, we revealed that the inoculant FZB42 could establish itself successfully in the rhizosphere without showing any durable effect on the rhizosphere bacterial community.

P55

Assessment of the microbial community in the cathode compartment of a plant microbial fuel cell

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In plant microbial fuel cells (Plant-MFCs) living plants and microorganisms form an electrochemical unit able to produce clean and sustainable electricity from solar energy. It is reasonable to assume that besides the bacteria in the anode compartment also the cathode compartment plays a crucial role for a stable high current producing plant-MFC. In this study we aim to identify dominant bacterial species in the cathode compartment of the plant-MFC.

DNA samples from the catholyte and a biofilm that formed at the cathode were prepared and graphite samples were fixed for fluorescent in situ hybridization (FISH). Bacterial 16S rDNA was amplified via PCR from all samples (650bp). With these amplicons 454-pyrosequencing was performed to identify the microbial key players in the cathode compartment. All sequences were assembled with 98% similarity by the Newbler software and the phylogenetic allocation of the 16S rDNA sequences was assessed by the ARB software. The obtained results allowed to select for specific 16S rRNA targeted oligonucleotide probes for FISH to verify the sequencing results and localize dominant bacterial species via confocal laser scanning microscopy (CLSM).

In the catholyte samples we found the genus *Brachymonas (β-Proteobacteria)* to be the most abundant in four out of six samples with the highest similarity to *Brachymonas denitrificans*. More striking was the abundance of a yet unknown member of the family *Sinobacteriaceae (γ-Proteobacteria)* in the biofilm samples from the cathode with a relative abundance of up to 30% of all sequences. These sequences show highest similarity to *Steroidobacter denitrificans*, but the entire family seems to be highly divers and not many species are described so far. From CLSM-observations the biofilm on the graphite samples appears to be sustained with a thickness of up to 17µm. Observations of bacteria in the biofilms with FISH support the data obtained by 454-sequencing.

P56

Phylogenetic clustering of *Bradyrhizobium* symbionts on host legume clades

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Introduction: -- The symbiosis of legume plants and nitrogen-fixing nodule bacteria constitutes one of the largest adaptive radiations in the biosphere, with more than 700 legume genera and a vast number of bacterial lineages. However, the extent to which bacteria are differentially associated with host clades at any level in the Family Leguminosae (genera, tribes or other defined monophyletic groups) is not well understood.

Objectives: To analyze host distribution and biogeographic structure in *Bradyrhizobium* nodule bacteria, 330 strains from 65 legume genera (in Australia, Asia, North and Central America, and Europe) were characterized.

Materials & methods: Portions of seven loci were sequenced and bacterial relationships were inferred by Bayesian analysis.

Results: Phylogenetic relationships for *nifD*, in the symbiotic island region of the *Bradyrhizobium* chromosome, conflicted substantially with a tree inferred for six housekeeping loci, suggesting widespread horizontal gene transfer. Permutation tests indicated that populations in most geographic areas were significantly differentiated, although major bacterial lineages were commonly distributed across multiple regions. After controlling for geographic variation, substantial disparities were found among legume clades in the extent of phylogenetic clustering of their associated *Bradyrhizobium* symbionts. Significant phylogenetic clustering was detected for symbionts on less than half of the legume genera. However, larger legume clades encompassing multiple genera in some cases utilized distinctive suites of bacteria.

Conclusion: These results indicate that *Bradyrhizobium* diversity is structured both by geography and by differential interactions with host taxa. Nevertheless, symbiont transfer to novel host clades has been a common event.

P57

Metagenome-sequencing of the obligate symbiotic *Mycoplasma*-related endobacteria of arbuscular mycorrhizal fungi

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Introduction: For more than 400 million years (My) arbuscular mycorrhizal fungi (AMF, *Glomeromycota*) form symbiotic associations with most land plants, for nutrient exploration and exchange. The obligate plant-symbiotic AMF host Gram-positive, uncultivable endobacteria traditionally called 'bacterium-like organisms' (BLOs). BLOs were found in all major evolutionary AMF lineages studied, but their function in the AM is unknown. 16S rRNA gene sequences of BLOs from diverse AMF lineages all fall in a monophyletic clade in the *Mollicutes* and within a single AMF spore 16S sequences are highly polymorphic (Naumann M, Schüßler A, Bonfante P (2010) ISME J 4: 862-71). Thus, BLOs represent an unnamed, diverse but monophyletic higher bacterial taxon. They colonize AMF and probably evolved endosymbiotically since more than 400 My.

Objectives: The aim of this study was to sequence the genetically probably polymorphic BLO genomes from three AMF, to gain understanding of their yet unknown functional role in the AM symbiosis.

Materials and methods: We isolated BLOs from spores of the AMF *Scutellospora heterogama* and *Diversispora epigaea*. Metagenomic DNA was used to prepare sequencing libraries with the Illumina's Nextera DNA Sample Preparation Kit. An additional library was prepared from DNA isolated from spores of *Geosiphon pyriformis* (*Glomeromycota*). The libraries were sequenced using the Illumina MiSeq platform.

Results and Conclusions: We assembled the preliminary metagenomes from BLO populations belonging to all three AMF studied. The metagenomes possess general characteristics related to the endosymbiotic lifestyle of BLOs, such as low G+C content and a relative high gene density. In addition, gene annotations revealed features that could be related to host-bacteria interaction, such as vitamin biosynthesis or signaling proteins.

By analyzing overlapping gene sets of BLOs from phylogenetically distant AMF we want to obtain important data about the BLO metagenomes, BLO evolution and the putative core-functions that BLOs play in the AMF symbiosis.

P58

Use of nonvertebrate animal model *Porcellio scaber* for *Clostridium difficile* fitness studies

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Terrestrial isopod *Porcellio scaber* (Crustacea) is widely used as a model organism in ecotoxicology. Molecular studies on *P. scaber* gut microbiota suggested that multiple anaerobic bacteria, together with *Clostridium difficile* can inhabit its gut. *C. difficile* is a spore-forming, toxin producing bacterium that is traditionally associated with human nosocomial intestinal infections. However, *C. difficile* can also be isolated from several other environments such as animal intestine, water and soil. Different *C. difficile* genotypes appear to predominate in different countries and/or different environments, which might be due to better bacterial fitness.

Our preliminary studies indicated that *P. scaber* could be useful model to compare bacterial fitness of different *C. difficile* strains *in vivo* conditions because it shows potential for *C. difficile* colonization and has high survival rates after exposure to nontoxigenic or toxigenic *C. difficile* strains. The objective of this study was to test *P. scaber* as a potential model for *C. difficile* fitness studies.

In order to determine *C. difficile* colonization in *P. scaber*, the number of ingested and excreted *C. difficile* colony forming units (CFUs) were compared. Animals were divided into groups of three and were fed with hazelnut tree leaves (*Corylus avellana*) for 5 days. Test groups were exposed to leaves with nontoxigenic *C. difficile* spores, and control group to leaves without any *C. difficile*. Fecal samples were collected daily and fecal *C. difficile* colony forming units (CFUs) were determined on selective, differential chromID *C. difficile* agar plates (bioMerieux).

In three out of six groups, the number of excreted *C. difficile* CFUs significantly differed from the number of ingested spores (4-8 fold difference), indicating multiplication of the bacterium. In other three groups, only slight difference in the number of *C. difficile* CFUs was observed. All control nonexposed animals tested negative. Based on these results, following studies are focused on the co-colonization experiments with two or more different strains.

P59

Exploring the bovine rumen bacterial community from birth to adulthood

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The mammalian gut microbiota is essential in shaping many of its host's functional attributes. One such microbiota resides in the bovine digestive tract in a compartment termed as the rumen. The rumen microbiota is necessary for the proper physiological development of the rumen and for the animal's ability to digest and convert plant mass into food products, making it highly significant to humans. The establishment of this microbial population and the changes occurring with the host's age are important for understanding this key microbial community. Despite its importance, little information about colonization of the microbial populations in newborn animals, and the gradual changes occurring thereafter, exists. Here, we characterized the overall bovine ruminal bacterial populations of five age groups, from 1-day-old calves to 2-year-old cows. We describe the changes occurring in the rumen ecosystem after birth, reflected by a decline in aerobic and facultative anaerobic taxa and an increase in anaerobic ones. Some rumen bacteria that are essential for mature rumen function could be detected as early as 1 day after birth, long before the rumen is active or even before ingestion of plant material occurs. The diversity and within-group similarity increased with age, suggesting a more diverse but homogeneous and specific mature community, compared with the more heterogeneous and less diverse primary community. In addition, a convergence toward a mature bacterial arrangement with age was observed. These findings have also been reported for human gut microbiota, suggesting that similar forces drive the establishment of gut microbiotas in these two distinct mammalian digestive systems.

P60

Computational approaches to microbial communication/quorum sensing signalling

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Introduction: Quorum sensing (QS) is a cell-cell communication mechanism which allows bacteria to coordinate gene expression via chemical signals. The arrangement of QS genes in bacterial genomes is diverse, and new combinations may appear with the rapid increase in the number of sequenced genomes. In current genomic databases QS genes are not consistently annotated, many of them are indicated as hypothetical proteins.

Objectives: Our goal is to review the arrangement of quorum sensing genes involved in acyl-homoserine lactone (AHL) signaling, which is one of the best characterized QS systems in Gram negative bacteria. The arrangement was defined as the relative orientation of QS genes (local topology) and also as positioning within the chromosome (chromosomal topology).

Methods: An automated computational pipeline was created for analyzing topological arrangements of AHL QS genes in over 2670 complete and 7702 draft bacterial genomes as well as 16585 Individual GenBank sequences. The system is implemented in Galaxy framework and relies on combinations of Hidden Markov Model recognizers developed for the various protein families.

Results: The analysis revealed 17 distinct gene-topology groups present in Proteobacteria [2]. We found that the genes within a given topology group are more related between species than between different topological arrangements found within the same organism.

Conclusions: Our findings suggest that the conservation of topological patterns between taxonomic groups are not random and there are unifying features among different topological patterns [1,2].

References:

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2. Gelencsér Z, Galbáts B, Gonzalez JF, Choudhary KS, Hudaiberdiev S, Venturi V, Pongor S: Chromosomal arrangement of AHL.driven quorum sensing circuits in Pseudomonas**.** *ISRN Microbiology* 2012, 2012**:**6**.**

P61

Characterization of a novel strain of the genus *Actinopolyspor*a, an extremely halophilic actinomycete isolated from Saudi Arabia

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Actinomycetes have attracted a great attention due to their ability for production of various useful secondary metabolites. . In these work, an extremely halophilic filamentous actinomycetes was isolated from hyper saline soil sample collected from Jeddah region in the west of Saudi Arabia. This isolate can grow at high NaCl concentration up to 30% (w/v). Phylogenetic studies using16S rDNA gene sequence analysis was performed in order to specify the isolate. Results showed that morphological, physiological and biochemical characteristics of the isolate were matched to the genus *Actinopolyspora*. Basing on phylogenetic studies and searching the isolate against the EMBL public database, the isolate is proposed as novel strain of the genus *Actinopolyspora*. The name *Actinpolyspora saudiensis* sp. nov. is proposed and the aim of the study was to describe the isolation, morphology, physiology and biochemical characteristics of this novel strain.

P62

Environmental conditions and community evenness determine the outcome of biological invasion

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Introduction: Biological invasion is widely studied, however conclusions on its outcome mainly originate from observations in systems that leave a large number of variables uncontrolled. This may lead to opposite conclusions on the role of a specific parameter, even in closely related ecosystems. Experiments conducted under controlled conditions give the opportunity to target some of these confounding factors, eventually explaining the inter-system variability.

Objectives: Using a fully controlled system, we evaluate the degree of invasion and the effect on the community functionality in relation to the initial community evenness under specific environmental stressors.

Methods: More than 3000 assembled denitrifying bacterial communities with different levels of initial evenness but with the same richness were created and incubated with and without salinity stress. The assembled community was challenged with a *gfp*-tagged, salt-resistant, non-denitrifying invader. After 20 hours of anaerobic incubation, the percentage of nitrite removal was used as a measure of functionality (i.e. denitrification). The invasion coefficient was determined by flow cytometry.

Results: In the absence of salt, invasion increased with an increasing Gini (Fig 1a). The invader affected the performance of the community by lowering the overall functionality, independent of the gini (Fig 1b) without influencing the growth of the community (Fig 1c).

In the presence of salt (Fig 1d), the degree of evenness did not influence the invasibility. However, the functionality was strongly influenced by the invader (Fig 1e). Under salinity stress and in the absence of invasion, nitrite was only partially reduced by the denitrifying communities with a high Gini. If the same communities were exposed to an invader, no negative correlation between functionality and the Gini coefficient was observed. The functionality was maintained at a high level over the complete range of evenness.

Conclusions: Under stress conditions an invasive species can preserve the indigenous functionality, while under non-stress conditions the functionality can be threatened. In the latter case evenness plays a crucial role in determining the community resistance to invasibility and in preserving ecosystem functionality.

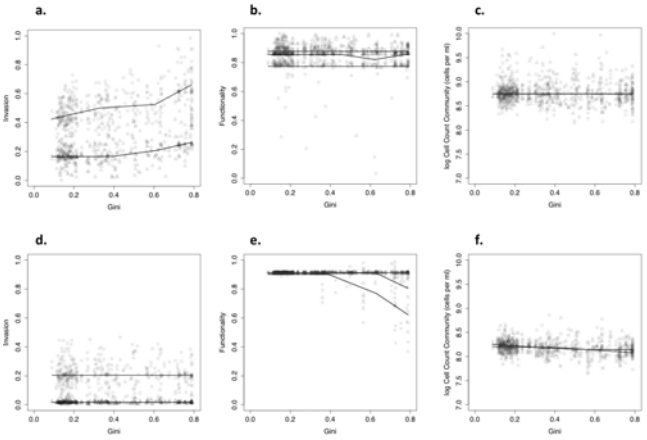

Fig 1. The individual plots show invasion (a and d), its effect on functionality (b and e) and the growth of the community (c and f) in relation to the Gini coefficient in the absence (a, b and c) and presence (d, e and f) of salinity stress.

P64

Genetic diversity of archaeal ammonia oxidizers drives potential nitrification rates in agricultural soils

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In this study we determined the abundance and diversity of AOA in four agricultural soils (two sandy and two clayey) during the growing season in 2010 using the functional gene *amoA* as marker. We also measured relevant chemical soil parameters and potential nitrification activity (NEA). Rates of NEA were significantly correlated to *amo*A gene abundance at all times (April, r^2 = 0.84; June, r^2 = 0.78; October, r^2 = 0.73), being significantly lower in soils with low pH and clay content compared to soils with high pH and clay content. To further understand the changes in community composition, we performed a barcoded pyrosequencing based on *amo*A. The AOA communities were found to be highly dynamic, with changes in community composition varying between 50% and 72% over time. The diversity estimates as well as the number of OTUs observed were higher in the soils with lower pH, compared to soil with higher pH. Clustering of the sequencing at cutoff of 90% amino-acid similarity resulted in 50 clusters with more than 10 sequences, which were spread over two known archaeal clusters, the Soil/Sediment and the Sediment/Soil cluster. In order to determine the extent to which genetic diversity could explain community functioning, we calculated a series of diversity measures based on OTUs. The average phylogenetic distance between OTUs was significant and positive correlated to the variation in the community functioning (r^2 = 0.38), indicating that more divergent communities were more productive. This percentage of variation increased (r^2 = 0.65) when using the Rao's index, taking into account both OTU distance and abundance. We also observed negative correlations between NEA and Shannon index or OTU richness, indicating that the most productive communities were dominated by few types. These results suggest that phylogenetic metrics based on the *amo*A gene can be used to predict changes in ammonia oxidation.

Moreover they indicate that few phylogenetically distant and abundant AOA-affiliated types are likely the responsible for NEA rates in these agricultural soils.

P65
Loss in microbial diversity affects nitrogen cycling in soil

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Microbial communities have a central role in ecosystem processes by driving the Earth's biogeochemical cycles. However, the importance of microbial diversity for ecosystem functioning is still debated. Here, we experimentally manipulated the soil microbial community using a dilution approach to analyze the functional consequences of diversity loss. A trait-centered approach was embraced using the denitrifiers as model guild due to their role in nitrogen cycling, a major ecosystem service. How various diversity metrics related to richness, eveness and phylogenetic diversity of the soil denitrifier community were affected by the removal experiment was assessed by 454 sequencing. As expected, the diversity metrics indicated a decrease in diversity in the $1/10^3$ and $1/10^5$ dilution treatments compared with the undiluted one. However, the extent of dilution and the corresponding reduction in diversity were not commensurate, as a dilution of five orders of magnitude resulted in a 75% decrease in estimated richness. This reduction in denitrifier diversity resulted in a significantly lower potential denitrification activity in soil of up to 4-5 folds. Addition of wheat residues significantly increased differences in potential denitrification between diversity levels, indicating that the resource level can influence the shape of the microbial diversity-functioning relationship. This study shows that microbial diversity loss can alter terrestrial ecosystem processes, which suggests that the importance of functional redundancy in soil microbial communities has been overstated.

P66
High rates of denitrification and nitrous oxide emission in biological soil crusts from an arid desert

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Using a combination of process rate determination, microsensor profiling and molecular techniques, we demonstrated that denitrification, and not anaerobic ammonium oxidation (anammox), is the major nitrogen loss process in biological soil crusts from Oman. Potential denitrification rates were 584±101 and 58±20 µmol N m^{-2} h^{-1} for cyanobacterial and lichen crusts, respectively. Complete denitrification to N_2 was confirmed in intact crusts by an $^{15}NO_3^-$ tracer experiment and proceeded at rates of 103±19 and 27±8 µmol N m^{-2} h^{-1} for cyanobacterial and lichen crust, respectively. Strikingly, N_2O gas was emitted at very high potential rates of 387±143 and 31±6 µmol N m^{-2} h^{-1} from the cyanobacterial and lichen crust, respectively, with N_2O accounting for 53-66% of the total emission of nitrogenous gases. Microsensor measurements revealed that N_2O was produced in the anoxic layer, and thus apparently originated from incomplete denitrification. Using quantitative PCR, denitrification genes were detected in both crusts and were expressed either in comparable (*nirS*) or slightly higher (*narG*) numbers in the cyanobacterial crusts. While 99% of the *nirS* sequences in the cyanobacterial crust were affiliated to an uncultured denitrifying bacterium, 94% of these sequences were most closely affiliated to *Paracoccus denitrificans* in the lichen crust. Sequences of *nosZ* gene formed a distinct cluster that did not branch with known denitrifying bacteria. Our results demonstrate that nitrogen loss via denitrification is a dominant process in crusts from Oman, which leads to N_2O gas emission and potentially reduces desert soil fertility.

P67
Sequence capture – a new tool for metagenomics

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NimbleGen sequence capture technology was mainly used for the enrichment and then sequencing of the human exome. On the other hand, metagenomic analysis of complex environments usually leads to a high number of small contigs and a high proportion of uncompleted genes. For instance, this proportion was estimated to 40% for the human microbiomes projects (MetaHit and HMP). As a test case, we applied sequence capture to improve the quality of a gene set obtained from a complex metagenome (a full scale anaerobic digester from a waste water treatment plant). Arachne assemblage of some 3 million Sanger reads (fosmid and plasmid end sequences) produced 7495 contigs organized into 2827 scaffolds, summing-up to 75.5 Mbp. From this assemblage, 75,488 non redundant genes were predicted, 10,785 of them being incomplete. Two million probes were designed from uncompleted genes sequences (as well as from unassembled fosmid end sequences) and were used for genomic DNA capture and subsequent 454 Titanium sequencing. Sequence assembly and analysis of an half 454 Titanium run validated the original Arachne assembly, improved its quality by linking together a number of contigs and by completing 6,575 out of 10,785 previously uncompleted genes. Moreover, an additional 25 Mb of contig's sequence was obtained. In conclusion, sequence capture is an efficient technology, complementary to metagenomic analysis of complex environment. It allows to obtain better assemblies and to establish non-redundant complete genes catalog. This technology could also be used for prokaryotic or eukaryotic genomic sequence finishing and resequencing genomes.

P68
Study of bacterial diversity in the topsoil and below the hardpan in an agricultural soil by metagenomics following by two analysis pipelines

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On earth, Bacteria are ubiquitous and even present in extreme environments (pH, temperature,...). In soils in particular, bacteria

are very abundant (up to 10^9 cells per gram of soil) but still poorly characterized. Thus, it is of paramount importance to use relevant study and analysis procedures to ensure that the results obtained closely reflect the real-life conditions. In the present work, we analyze the bacterial diversity in the topsoil and below the hardpan in an agricultural soil using the metagenomics approach, with the Ion Torrent PGM sequencer. The soil samples was collected at three depths : 10 cm (topsoil), 25 cm (topsoil above the hardpan) and 45 cm (below the hardpan), in a tilled and a no tilled plot. The taxonomic analysis of the reads obtained are carried out according to two different procedures with the RDP classifier program and with a confidence score threshold of 0 and 0.99. The 0 threshold is used to assign a species to all reads, each read being therefore assigned to its most closest known species. The threshold of 0.99 enables us to focus on reads being assigned to a species with a high degree of confidence. In this case, each read is assigned to the most specific rank having a confidence score higher than 0.99. The bacterial diversity was then compared between the different conditions. Results obtained demonstrate that the bacterial communities were not the same in the two horizons. For example, some classes of *Acidobacteria* were up to 11 fold more numerous in topsoil while others was until 12 fold more represented below the hardpan. The biomass and the bacterial diversity (Shannon index) were also greatly different between the two depths.

P69
16S rRNA gene variability in bacterial genomes and its consequences for bacterial community analyses

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Although 16S ribosomal RNA gene represents the most used target of study in bacterial ecology, its use for the description of bacterial diversity is limited because of variable copy numbers in bacterial genomes and sequence variation within closely related taxa or even a single genome. Here we used the information from sequenced bacterial genomes to explore the variability of 16S rRNA sequences and copy numbers at various taxonomic levels and applied it to estimate bacterial genome and DNA abundances. In total, 7,081 16S rRNA sequences were *in silico* extracted from 1,690 available bacterial genomes (1-15 per genome). While there are several phyla containing low 16S rRNA copy numbers, in certain taxa, e.g., the Firmicutes and Gammaproteobacteria, the variation is large. Genome sizes are more conserved at all tested taxonomic levels than 16S rRNA copy numbers. Only a minority of bacterial genomes harbors identical 16S rRNA gene copies, and sequence diversity increases with increasing copy numbers. While certain taxa harbor dissimilar 16S rRNA genes, others contain sequences common to multiple species. Sequence identity clusters (often termed operational taxonomic units) thus provide an imperfect representation of bacterial taxa of a certain phylogenetic rank. We have demonstrated that the information on 16S rRNA copy numbers and genome sizes of genome-sequenced bacteria may be used as an estimate for the closest related taxon in an environmental dataset to improve the estimates of the relative abundance of individual bacterial taxa in environmental samples (Větrovský and Baldrian 2013). Using an example from forest soil, this procedure increased the abundance estimates of Acidobacteria and decreased these of Firmicutes. Using the currently available information, better estimates of bacterial community composition can be obtained if the variation of 16S rRNA copy numbers among bacteria is considered.

Větrovský T, Baldrian P (2013) PLoS ONE 8: e57923.

P70
Zooming the assemblies of microbial communities interacting with sugarcane

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Sugarcane is one of the most important agricultural crops, being cultivated in over 110 countries worldwide. Brazil is the largest producer, presenting the São Paulo State as the major source of this material used as source for sugar, ethanol and renewable energy, where it is could be crucial to reduce CO_2 emissions. In the context of high production crops, lower expansion and reduced environmental impacts, the microbial community plays a crucial role in plant development. Therefore, this study aimed to zoom in and zoom out our view on the microorganisms interacting with sugarcane plants. In the zoom in approach we evaluated CO_2 effects in rhizosphere communities by Stabel Isotope Probing (SIP) and the dependency of the mycorrhizal association on the bacterial diversity of soils was examined by the dilution-to-extinction approach. Our results showed the selectiveness of differential bacterial groups in plants cultivated under distinct conditions of CO_2, and the role of rare groups was evidenced by the lowest mychorrization in sugarcane plants cultivated in soils with lower values of microbial diversity. In our zoom out analyses, the occurrence of biogeographical patterns of bacteria and fungi in sugarcane was evaluated. By contrasting such spatial effect with those related to the cultivation techniques, it was observed a significant effect of location in the assembling of such communities. Together with, the most important factor explaining the variation in the structures of such communities were the application of vinasse (due to the elevate concentration of potassium), the contents of organic matter, and the soil texture (mainly driven by the clay contents). These results are the first insights that rely on a wider view of microbes present in the sugarcane fields.

P71
Analysis of bacterial communities in Lake Kivu by comparative DNA- and RNA-based pyrosequencing

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Meromictic Lake Kivu in East Africa is a unique ecosystem with one of the largest CH_4 reservoirs in the world. The lake has a permanent density stratification that separates an oxic and low nutrient content mixolimnion from an anoxic monimolimnion that is rich in dissolved salts, carbon dioxide, methane (CH_4) and nutrients. However, CH_4 concentrations in the mixolimnion are surprisingly low compared to other lakes globally indicating an intense CH_4 oxidation. We initially showed vertical stratification of

the bacterial community composition (BCC) in the water column with some species specific to certain layers and core abundant species being more stable over time than rare ones, which might indicate possibly active rare species. The response of the total community to biotic and abiotic factors depends on whether individual phylotypes are active or not. Therefore, the temporal and vertical fluctuations of total vs. active BCC were further investigated by DNA and RNA based 16S pyrosequencing in two sampling campaigns in 2012. In parallel, a large set of environmental parameters were measured.

Clustering analysis revealed significant and systematic BCC shifts with depth and depending on whether DNA or RNA was extracted. Besides, rare species in high proportion specific to either active or total community were found, indicating the existence of an active rare biosphere and the possible underestimation of phylotypes by use of only one nucleic acid pool.

At phylum level, total and active BCC were significantly different in mixolimnion and transition zone. In the surface water, *Actinobacteria* were found highly dominant, but not active, whereas *Cyanobacteria* were active and abundant in the photic layer. In the suboxic layer, highly abundant active *Nitrospira* were observed. The relative abundance of active *Methylococcales* and abundance of *pmoA* gene (aerobic methane oxidizers) were correlated in the transition zone. Aerobic CH_4 oxidation might be the main process preventing CH_4 from escaping to the atmosphere. The co-occurrence of active sulfur-oxidizing and sulfate-reducing bacteria in the monimolimnion may suggest an internal sulfur cycle.

In conclusion, these results allow us to identify the main bacterial players driving the major biogeochemical cycles in this unique tropical lake and understand their ecology.

P72

Skewing the trophic structure of bacterial and fungal communities through sequential enrichments involved in lignocellulose and furanic compound bioconversion

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In order to develop a targeted soil metagenomics approach to prospect hemicellulases and enzymes involved in the bioconversion of furanic compounds using biased microbial communities, we constructed two sequential enrichment cultures using wheat straw and torrefied wheat straw as carbon sources in aerobic conditions. We observed exponential growth (using cell counts), indicating that microbial communities were growing on the substrate. The abundances (16S rRNA gene copy numbers/ml) of the bacterial communities were between *log* 8.7 - 9.5. However, the abundance of fungal communities showed a greater variation, with high abundances in transfers 4 and 7 (approximately *log* 8.0 ITS copies/ml) . The selected microbial consortia reached structural stability after six transfers. We observed that the structures of the fungal communities were influenced by the substrate, as observed by PCR-DGGE. From both enriched cultures, we isolated some fungal (*Coniochaeta ligniaria* and *Plectophaerella* sp.) and bacterial strains (*Klebsiella oxytoca*, *Acinetobacter calcoaceticus* and *Pseudomonas fluorencens*) with high lignocellulosic activity in the cellulose and hemicellulose proxies carboxy methylcellulose (CMC) and xylan. Moreover, some bacterial isolates were able to grow on as well as degrade 1mM of furanic compounds (5-HMF and furfural). The analysis of bacterial succession and composition was achieved by 16S rRNA gene amplicon sequencing performed with some of the enriched cultures. Most taxa thriving in the lignocellulosic mixed cultures (or involved in bioconversion of furanic compounds) belonged to the orders Enterobacteriales, Pseudomonadales, Xanthomonadales, Flavobacteriales and Sphingomonadales orders. These biased cultures are the starting points for metagenomic library construction and functional screenings.

P73

Using a metagenomic approach to explore microbial community structure and function in Arctic snow

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The Arctic seasonal snowpack can extend at times over a third of the Earth's land surface. This chemically dynamic environment interacts constantly with different environmental compartments such as atmosphere, soil and meltwater and thus, strongly influences the entire biosphere. However, the microbial community associated with this habitat remains poorly understood. Here, we applied a metagenomic approach to explore community structure and function in Arctic snow. Samples, collected at different times, depths and with variable chemical composition, were compared in order to determine whether functional community properties shifted in snowpacks. Globally, *Fungi*, *Bacteroidetes*, *Proteobacteria* and *Cyanobacteria* were predominant in metagenomic datasets harboring over 900 thousand reads (14.62 Mb), but changes in community structure were apparent throughout the field season with an increase in reads related to Fungi between the 25th of April and the 27th of May. On the 20th of May, when the snowpack started melting, we also observed a decrease in reads related to *Cyanobacteria* and an increase in those related to *Firmicutes* and *Streptophyta*. The majority of reads were unassigned to specific metabolic categories in all snow metagenomes (varying anywhere between 58 and 88% of reads), which highlights the lack of related functional data in databases. Of the reads identified, most were associated with carbohydrates (10-19%), virulence (8-17%), amino acids (8-12%), protein metabolism (4-9%), DNA metabolism (4-8%), cell wall and capsule (5-7%), cofactors, vitamins, prosthetic groups, pigments (4-7%) and respiration (3-9%). Sequence reads were also associated with pigment biosynthesis, osmotic and oxidative stress response, and cryoprotectants, which may signal how microorganisms cope with the harsh conditions (such as intense UV radiation and cold temperatures) characteristic of Arctic snowpacks. Our study provided new insights into microbial life in Arctic snowpacks, shedding light on the possible impact of microorganisms on geochemical cycling.

P74

Understanding bacterial response to environmental perturbations at multiple spatial scales using a metagenomic approach

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Soils are among the most microbial diverse ecosystems of the Earth. Despite considerable sequencing of DNA and rRNA from different soils, much remains to be explored in terms of how these communities are structured, the extent of their interactions and their role in ecosystem functioning. The spatial distribution of bacterial communities inhabiting the soil shows high heterogeneity at different scales, but is still almost unexplored. Some studies have attempted to link the spatial diversity of soil microbes with soil physicochemical parameters (*e.g.*, relationship between soil pH v and *Acidobacter* abundance). By using various metagenomic tools, such as phylogenetic microarrays and metagenomic sequencing, we studied the spatial distribution of bacteria in a soil core (30cm diameter) at different spatial scales in order to understand the relationship between genetic diversity of microbial communities, spatial distance and soil physicochemical parameters. Half of the core was contaminated by hydrocarbons prior to distance-based sub-sampling. The result of metagenomic analysis provided evidence of the relative importance of spatial distance and hydrocarbon pollution. Both bacterial phylogentic diversity and community function were evaluated. Initial spatial modeling of the soil contamination, soil physicochemical characteristics and microbial community distribution using a geographic information system (GIS) and geostatistical tools quantified the relative importance of the different parameters. This spatial approach was also applied to larger-scale structures, such as Chilika Lake in eastern India.

P75

Determination of soil microbial community structure and respiration during soil cooling

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Question: Field-experiment and modelling studies have concluded that global warming will impact on soil microbial communities, and a long-term warming experiment showed the decline of microbial respiration. A major concern is to determine if this decline is the result of loss of the majority of labile organic matter (LOM) or down-regulation of microbial activity due to the change in temperature (acclimation). Moreover, if community structure is highly affected by temperature, this could lead to a reduction in microbial respiration (enhancement).

Methods and Results: To address this question, soils associated with different ecosystems were subjected to soil-cooling. This method permits discrimination between these hypotheses by reducing the loss of LOM in cooled soil versus a control condition. We measured the respiration rate of fourteen soils sampled from temperate, Mediterranean or tropical regions and most did not respond significantly. Acclimation responses were, however, found in pine forest and agricultural soils (England) and the opposite results were obtained for two tropical soils (acclimation versus enhancement). An Italian forest soil also showed enhancement but at the lower ranges of temperature tested. Microbial biomass was determined by quantitative PCR (qPCR) targeting 16S rRNA genes (Bacteria and Archaea) or internal transcribed spacer (ITS, fungi) and community structure was characterised by multiplex-terminal restriction length polymorphism (M-TRFLP).

Conclusions: Although, community varied significantly between the different treatments for each soil, biomass and respiration few were affected, suggesting functional redundancy in carbon mineralisation. These results are now being assessed, with further data and enzymatic assays, to select soils for more detailed analysis of community composition.

P76

Metatranscriptomics of microbial communities from mangroves

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Mangroves compose a biome specific in the interface between the continent and the ocean in intertropical regions. Such location creates unique environmental conditions, especially for salinity, frequent anaerobiosis and seasonal changes in nutrient availability. The present study was to examine, by metatranscriptomics, the functioning of microbial communities in four mangroves areas located in the State of São Paulo, with different contamination, BrMgv01 (low oil contamination), BrMgv02 (high oil contamination) BrMgv03 (anthropogenic contamination) and BrMgv04 (pristine mangrove). The taxonomic and functional approaches were based on MG-RAST automated annotation with cut off similarity 60% and E value of 1^{-5} against the RDP and N5MR databases. The RNA was extracted from sediment samples and further sequencing using a large-scale strategy (Illumina HiScan), allowing the robust comparison of genes expressed in mangroves under different states of preservation. The sequencing effort resulted in approximately 140.52 Mb (for all areas - BrMgv01 to 04). The first analysis revealed the dominance for domain Bacteria (92-97%), majorly represented by Deltaproteobacteria (11-13.8%) and Gammaproteobacteria (8-13.8%). Within the domain Archaea (0.3-1.7% of total sequences), the dominance was observed for Euryarchaeota, but the BrMgv03 presented a remarkable abundance of Thaumarchaeota. The microbial core involved in methane, nitrogen, and sulphur metabolism consists mainly of Syntrophobacteriaceae (Deltaproteobacteria), Hydrogenophilaceae (Betaproteoobacteria), Xanthomonadaceae (Gammaproteobacteria), Vibrionaceae (Gammaproteobacteria) and Nitrospulmiliaceae (Thaumarchaeota). In sum, information

was obtained on these functional communities, not only describing a gene, but also its expression in the mangroves, and was different for each mangrove and differed also of its metagenomics, contributing therefore to a better understanding of the ecological dynamics in this ecosystem.

P77

Metagenomic discovery of rhizosphere microbiome dynamics from mandacaru in Caatinga

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The caatinga is a tropical dry forest located in the northeastern Brazil. It is characterized by periods of drought, high temperatures and seasonal rainfall. In the last years this biome has been threatened by desertification and climate change. While the flora, which is dominated by xerophytes, is well known, there is no or few information on microbial communities associated to these plants. The aim of this study is to access the rhizosphere microbiome associated with mandacaru (*Cereus jamacaru*), a native and typical cactus of this biome. For this, we sampled rhizosphere soil from three different plants of mandacaru located in Petrolina, Brazil. The same three plants were sampled during the rainy and during the dry season in order to identify the community structure fluctuation over the two distinct seasons. The metagenomic DNA isolated from rhizosphere samples was used to construct six libraries (3 plants X 2 seasons); and we used the Ion Torrent (PGM) for shotgun sequencing. We generated approximately 240 million sequences with an average length of 100 bp, which enabled us to access the microbiome structure and functions in the rhizosphere of the mandacaru. The data were uploaded to the MG-RAST server for annotation and STAMP was used for statistical analyses. Most of the sequences were from Bacteria (93.3%), followed by Eukaryota (5.2%) and Archaea (1.4%). The bacterial community was dominated by Actinobacteria, Proteobacteria and Acidobacteria phylum. While Acidobacteria, Cyanobacteria and Bacteroidetes were significatively more abundant in the rainy season, Actinobacteria was more abundant during the drought period. Preliminary analysis indicated that genes related to virulence, disease and resistance functions were more abundant in the dry season, while genes related to the nitrogen metabolism were more abundant during the rainy period. Further data analysis is being conducted to properly understand the rhizosphere microbiome dynamics in mandacaru.

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P78

Assessment of the effects of genetic modified (GM) maize cultivation on ammonia-oxidizing bacterial and archaeal communities

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Over the last decade, studies using soil-borne microbial communities as indicators of soil quality have greatly contributed to the knowledge about the impact of GM plant cultivation in the environment. However, much is yet to be learnt about this field. Therefore, the use of functional communities as bioindicators was proposed here to further investigate the subject. With this purpose, we proposed to detect any possible modification in ammonia-oxidizing communities related to GM maize cultivation. Our results showed a significant fluctuation in abundance values of both ammonia-oxidizing bacterial and archaeal communities related to GM maize genotype. Moreover, plant genotype, soil type and cultivation season considerably influenced the abundance of these communities. When the structure of the ammonia-oxidizing communities was analyzed, a different pattern was observed from abundance. It was possible to observe different patterns in DGGE profiles related to abiotic factors, such as soil type and GM maize cultivation season, but not to plant genotype. The abundance of ammonia-oxidizing communities was proved to be more responsive than the structure in the detection of modifications in the rhizosphere microbial communities related to GM maize. However, the fluctuation in abundance values was punctual and transient, probably not representing a significant modification in soil functioning. We concluded that abundance of ammonia-oxidizing communities is a sensitive parameter that could be used for effect detection in GM plant cultivation.

P79

Survival and adaptation of introduced phosphate-solubilizing bacteria (PSB), *Bacillus sp.* during phytoextraction of Cd-contaminated soil

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Phytoextraction is considered as an emerging technology for the removal of heavy metals from polluted soil. Recently, it has been focused on the application of plant growth promoting rhizobacteria (PGPR) to improve the efficiency of phytoextraction. This study was conducted to investigate whether or not phosphate-solubilizaing bacteria (PSB) as a kind of PGPR, enhanced the uptake of Cd by plant and how the soil microbial community of the introduced bacterium and indigenous soil bacteria changed during phytoextraction. *Brassica juncea* was planted in a pot containing soil artificially contaminated with Cd (38.8±1.45 mg/kg) and *Bacillus sp.* was inoculated as PSB. Each pot was re-inoculated with the same bacterium on 1st, 2nd, 4th and 6th week to maintain a sufficient bacterial population. An uninoculated pot was used as control. For analysis of soil microbial community, DNA was extracted from soil at time intervals, and the DNA samples were analyzed by pyrosequencing with a Roche/454 GS FLX Titanium platform (Chunlab, Inc., Korea). The major phyla in initial Cd-contaminated soil were Proteobacteria (35%), Actinobacteria (38%) and Firmicutes (8%). While Proteobacteria were dominant on 2th and 6th week (41 and 54%, respectively) in inoculated soil, Firmicutes dramatically increased, contributing 63% of the sequences found in 8th-week soil, and they were mainly belong to Bacilli class (61%). For the unioculated soil, the proportion of α-Proteobacteria increased after 8 weeks. Interestingly, Actinobacteria class, which was

originally present in the soil, seemed to disappeared during phytoremediation irrespective of whether PSB was inoculated or not. Heat map and hierarchical cluster analysis revealed that the inoculated soil on 8th week was found to be completely separated from the other soil samples due to the dramatic increase of *Bacillus aryabhattai*. Principal component analysis showed that the microbial diversity in the 8-week inoculated soil was clearly shifted and formed a distinct cluster compared to the other soils. *Bacillus sp.* accounted for more than half of the total bacteria community in soil, which resulted in the increase in the dominance (Simpson index: 0.148) of the community and the decrease in the richness and diversity (Shannon index: 3.835). These findings revealed that it took at least 8 weeks for the inoculated Bacillus sp. to functionally adapt to the introduced soil against competition with indigenous microorganisms in soil.

P80

The rhizosphere microbiome of potato grown at high altitudes in its center of origin, the Central Andean highlands

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Introduction: The Central Andean highlands, confining Ecuador, Peru and Bolivia, are the origin of potato diversity, covering more than 3,000 varieties. Potato is cultivated in these regions up to more than 4000 m a.s.l and plant-associated microorganisms, which are known to play an important role in plant nutrition and health, might be particularly important under these extreme conditions.

Objectives: Our aim was to study the diversity and functional potential of the potato microbiome of potato grown in the highlands of Ecuador, Peru and Bolivia grown under different geographical and seasonal conditions in detail. We furthermore developed new primer systems to appropriately address the abundance and diversity of *Alpha-* and *Betaproteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*.

Materials & Methods: Potato plants and attached rhizospheres were sampled from fields at four altitude levels in each country, at three plant development stages. Diversity and abundance of rhizosphere bacteria was analyzed using 454 16S rDNA amplicon sequencing and phylum- and class-specific T-RFLP and qPCR of the most abundant bacterial taxa. In this context, new phylum- and class specific 16S PCR primers were developed. Bacterial endophytes were isolated from roots of flowering plants, phylogenetically characterized and screened for plant beneficial characteristics.

Results: The diversity and quantity of rhizosphere bacteria under the influence of soil chemistry, geographic and seasonal parameters were assayed using DNA based molecular methods, qPCR, T-RFLP analysis and 454 sequencing. Multivariate data analysis provided us insight into the shaping factors of the bacterial community composition. Plant-beneficial features could be attributed to a large percentage of isolated endophytes, with some of them showing positive results in multiple assays.

Conclusions: The potato rhizosphere microbiome was affected by agricultural management and environmental conditions. The assessment was facilitated by novel PCR primers targeting important and abundant microbial taxa.. A better understanding of the ecology of plant-associated microorganisms and the identification of bacterial strains showing plant-beneficial functions could contribute to develop strategies warranting sustainable potato production.

P81

Diversity and evolution of the microbial communities at the rhizosphere of *quercus ilex* subsp. ballota after a wildfire

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Wildfires, as occasional events of natural origin, have probably influenced the vegetation of the Mediterranean basin before the presence of man in this area. This influence together with others factors, like regular droughts, elevated temperatures or pasturage during thousands of years, has allowed the development of a specific and adapted vegetation. This is the case of the holm-oak (*Quercus ilex* subsp. ballota) that possesses the capacity of re-sprout after the fire. In Spain the holm-oak forests are scarce due to their management to a pastoral woodland called dehesa, where fire and clear-cutting cannot be excluded from its management. In order to recover these plant formations after a wildfire it is important to understand the processes that affect the recovery of the soil microbial communities since they positively influence soil fertility and plant growth.

On September 2005 a wildfire happened in the National Park of Sierra Nevada, southeast of Spain, which affected to 3,416.74 ha, with the presence of holm-oaks in 412 ha of the total burned area. We are studying the prokaryotic diversity associated to the holm-oak rhizosphere 3 and 6 years after a wildfire at 4 different zones, with 3 sites at each zone. As a first approach, the bacterial diversity was analyzed by Temperature Gradient Gel Electrophoresis fingerprinting by the use of universal primers and specific primers for groups such as a-Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes. The phylum Actinobacteria showed differences for these communities between burned and unburned soils, both 3 and 6 years after fire. The second approach was the pyrosequencing of 441,561 amplicons of the 16S rRNA gene, with an average of more than 16,000 reads per replicate. The data showed us that Proteobacteria is the most abundant phylum (more than 22 % of the sequences), followed by Actinobacteria (16.7 %), Acidobacteria (6.5 %), Bacteroidetes, Verrucomicrobia, Planctomycetes, Gemmatimonadetes, Chloroflexi and Firmicutes. But at the burned zones the phylum Actinobacteria reached 42.5 % and 25.2 %, 3 and 6 years after the wildfire, with a statistically significant difference respect to the unburned zones. Thus, this phylum is proposed as biomarker to follow ecosystem recovery. The results will be discussed at the presentation.

P82

Introducing *Candidatus* Nitrosopumilus halotolerans – a "rare" and monophyletic thaumarchaeon thriving in the brine-seawater milieu of Red Sea's deep-sea brine pools

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The Red Sea is home to over 25 deep hypersaline anoxic basins (DHABs) along its central axis that are geochemically distinct and characterized by dynamics of salinity and concentrations of electron acceptors/donors along the brine body. The brine-seawater interface (BSI) layer is a particularly interesting environment, where the concentrations of metabolites (CH_4, NH_4^+, H_2S, and heavy metals) are higher than in the overlying seawater (OS) and steep physicochemical gradients (e.g., O_2) are present, thereby providing a suite of niches for various chemolithoautotrophs emanating from the OS. Given that the BSI is metabolically more active than the boundary layers and the fact that ammonia-oxidizing Archaea (AOAs) predominate the oceanic deep-sea environment, we investigated how the *in situ* settings of the BSI has impacted on their diversity and genetic differentiation using phylogenomic-based approaches. The composition of 16S rRNA and three other Archaeal-associated functional genes in the brine-seawater interface (5.2-18% salinity) of five geochemically distinct DHABs were predominated by a single thaumarchaeon. This was phylogenetically discrete from those in the overlying seawater (50-1500 m), but virtually identical to the type strain, *Nitropumilus maritimus*. Surprisingly, ammonia oxidizing Bacteria were absent despite the favorably high concentrations of ammonia in view of the differences in uptake kinetics and the general sensitivity of AOAs to ammonia. Comparative genomics of five single-cell amplified genomes and metagenomes of two highly enriched AOA cultures further indicated that the monophyletic thaumarchaeon is a novel species, which we provisionally refer to as *Candidatus* Nitrosopumilus halotolerans. Although ~75% of its proteome resembles that of *N. maritimus*, the genome carries several unique genes of unknown functions and interestingly also a few extra genes associated with cell wall/membrane biogenesis, DNA repair, replication and recombination, and defense mechanism. The oxidation of ammonia by chemoautotrophic prokaryotes is a prime example of processes that are contrained even at intermediate salt concentrations (up to 94 g Cl l^{-1}). Hence, the predominance and ability of *Ca.* Nitrosopumilus halotolerans to thrive in sulfide-rich and ammonium-replete hypersaline environments may have provided this thaumarchaeon a competitive edge in suboxic habitats.

P83

The white rot fungus *Phanerochaete chrysosporium* structures the diversity of the associated bacterial community during wood decay

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Introduction: Wood recycling, a key process in forest biogeochemical cycles, is mainly driven by microorganisms. Among them, saprotrophic basidiomycetes, and particularly white rot fungi, have been well described as the most important wood decomposers. Much less is known about the diversity of bacterial communities that co-inhabit with these fungi on decaying wood. The concept of the mycosphere effect defines the impact of fungal cells on bacterial communities. Although it has been highlighted for many fungi with very distinct ecology, like symbiotic, phytopathogenic and food processing fungi, this concept has been poorly investigated for white rot fungi.

Objectives: The aim of our study was to determine whether a white rot fungus can shape bacterial communities during the wood decay process, and how these bacterial communities evolve on temporal basis.

Methods: The experiment was designed at microcosm scale and used an enrichment method. A microbial suspension extracted from a forest soil as a main inoculum, was added to microcosms containing sawdust as the growth matrix, with or without the white rot model *Phanerochaete chrysosporium*. Three enrichment steps were performed. To monitor the succession of bacterial communities for these two conditions, a 16S rRNA amplicon pyrosequencing approach was used.

Results: Based on a good sampling coverage, richness estimation revealed a much higher richness in the bacterial community extracted from the forest soil microbial inoculum than in the bacterial communities evolving in sawdust microcosms, demonstrating that sawdust behaved as an environmental filter. In contrast, a very similar richness was observed in the microcosms over time. Based on statistical analyses (ANOSIM, SIMPER), differences between bacterial communities associated or not with the fungus were detected for the first step of the enrichment. During the course of the enrichment process, dissimilarity between replicates and between treatments increased, but with a lower dissimilarity in presence of *P. chrysosporium*, suggesting that the fungus structured the taxonomic diversity of the bacterial communities. Few discriminating OTUs, all belonging to *Proteobacteria* phylum and with high relative abundance, explained the main differences between treatments.

Conclusion: These results demonstrated a mycosphere effect of *P. chrysosporium* on wood associated bacterial communities, notably characterized by a selection of *Proteobacteria*.

P84

Soil aggregates – protective hot spots for microbes against oxidation

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Soil aggregates are the basic structural unit of soil with strong impact on soil functionality. Their spatial arrangement establishes special microhabitats with proper microbial community structures and functions. It has been reported that aggregates can protect from oxidation (carbon mineralization) both recalcitrant SOM (lignin, humic acid and alkyl C) and labile SOM (polysaccharides and proteins) and that most of stabilized soil C is located into the smaller aggregates. Concerning the SOM preservation inside aggregates and protection against environmental impacts, in the present study we focused on the effects of SOM oxidation on autochthonous microflora in terms of soil microbial biomass (amount of double strand intracellular DNA) and microbial community composition.

Three different calibrated sizes of aggregates (0.5-1mm, 500-100µm and <100µm) were obtained by dry sieving from 2mm sieved fraction from a natural soil. Each fraction of aggregates was subjected to Low Temperature Ashing (LTA) by oxygen

plasma treatment at various time period of exposure (0, 5, 20 and 48 hours). This technique allows the removal of organic material by means of the oxidation at low temperature without altering the aggregate fabric and the mineral fraction. The protective effect of aggregates has been assessed on the basis of the amount of recovered DNA in the aggregates after LTA treatment. Sequential extraction was performed to discriminate between the extracellular and intracellular fractions of the soil metagenome, followed by comparative community level genetic fingerprinting (PCR-DGGE) of archaea, bacteria and fungi. The protective effect of aggregates has further been assessed as amount of extracellular DNA.

We obtained evidences that aggregates can not only protect intracellular DNA of all microbial domains but also soil extracellular DNA, this latter with evolutionary implication. This study contributes to molecular level understanding of oxidation and protection of soil DNA in soil aggregates.

Future studies will be addressed on analysis of β-glucosidase activity and detection of β-glucosidase encoding genes in these aggregates, as extracellular enzymes are actively protected within microaggregates.

P85
Bacterial succession in a developing salt marsh ecosystem

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Salt marshes are boundary landform ecosystems located at the edge of the land and sea. At the island of Schiermonnikoog (NL), the daily influence of tidal movements result in the deposition of silt and clay particles on the marsh surface. As a result, the island slowly extends seaward side, forming a chronosequence, which spans hundreds of years of succession. In this study, we combined spatial-temporal sampling [four sampling times along five successional stages - 0, 5, 35, 65 and 105 years of development (yd)] with 454-pyrosequencing of the bacterial 16S rRNA gene, to investigate successional assemblages of bacterial communities (BC) and to test how environmental factors [soil physical structure, sodium (Na), dissolved organic carbon (DOC), sulphate (SO_4) and pH] were associated with observed changes in community structure. Overall, nutrients and salinity increased over time, reaching a plateau at later stages of succession (65-105 yd), accompanied by a slow but significant decrease in pH. We observed that BCs were different in each successional stage using both taxonomic (P≤0.001) and phylogenetic composition (Unifrac). Community dissimilarity was highest at 5-35 yd, whereas phylogenetic distance decreased over the course of succession. Analyses performed at phylum level revealed that the largest differences were due to the linear decrease of Cyanobacteria (3.85±0.78% to 0.1±0.06%) and Verrucomicrobia (5.25±0.99% to 1.2±0.30%), followed by an increase of Chloroflexi (2.6±1.13% to 16.95±1.70%). Changes in BCs along the chronosequence were significantly correlated with soil physical structure (silt, clay and sand), as well as chemical element content (Na, SO_4, DOC; P≤0.001). Seasonal effects in BC structure were observed only at initial stages (0-5 yd) with no physico-chemical parameter explaining such changes. In summary, our results suggest that high rates of immigration might be involved in the BC assemblages at initial stages of succession, while at later stages the increase in nutrient partitioning (niche establishment) might act as a selective effect (determinism), driving the more constant BC assemblage (lower turnover). Taken together, our data highlight the ecological processes as well as environmental parameters that alter the fate of the BC assemblages in a natural soil chronosequence.

P86
Microbial biogeography in Arctic snowpacks

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The Arctic environment is undergoing changes due to climate shifts, receiving contaminants from distant sources and experiencing increased human activity. Climate change may alter microbial functioning by increasing growth rates and substrate use due to increased temperature. This may lead to changes of process rates and shifts in the structure of microbial communities. Biodiversity may increase as the Arctic warms and population shifts occur as psychrophilic/psychrotolerant species disappear in favor of more mesophilic ones. In order to predict how ecological processes will evolve as a function of global change, it is essential to identify which populations participate in each process, how they vary physiologically, and how the relative abundance, activity and community structure will change under altered environmental conditions. We used metagenomic tools such as phylogenetic microarrays and high throughput sequencing to explore microbial community structure in samples collected from various Arctic snowpacks (North Pole, Greenland and Ny-Alesund) at different seasons (spring, summer, winter). The results from this study offer insights into the mechanisms that generate and maintain diversity, such as speciation, extinction, dispersal and species interactions.

P87
Metaproteogenomic insights of contaminated microbial communities in marine and freshwater environments

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Background: The identification of key actors in microbial communities is a hard task, particularly for metal-contaminated sediments. Indeed, cultured microorganisms often do not have important roles in situ and PCR-based approaches focused on one gene such as the 16S rRNA cloning/sequencing and pyrosequencing present many biases. As a result key bacteria are frequently overlooked.

Objectives: The objective of the present research was to perform a functional metaproteogenomic shotgun analysis of two anthropogenically influenced metal-contaminated sediments in order to identify the key players in the top sediment layer and propose a model for the functioning of the community.

Methods: Contaminated sediments were sampled in Station 130 in the North Sea (Belgium, As contamination) and in Férin (France, Pb and Zn contamination). For shotgun sequencing DNA was purified and sequenced on a GS-FLX pyrosequencing platform. Sequences were then assembled and contigs were submitted to the MG-RAST pipeline. For metaproteomics total proteins were separated by 1D SDS-PAGE and cut into gel slices. After trypsin

digestion peptides were analyzed using MS/MS nano-LC mass spectrometry (TripleTof 5600, ABSciex).

Conclusions: The abundance-rank curves obtained were almost flat and indicate that many species may be considered key elements in the two sites. The most abundant genera never exceeded 3% of the sequences. The marine station 130 was dominated by three genera (*Pseudomonas, Shewanella, Congregibacter*) together with *Nitrococcus* and *Alkalilimnicola*. A link with arsenic effluxes from sediments was established. The riverine station contained *Burkholderia* and *Pseudomonas*, with other bacteria such as *Leptothrix* and *Methylibium*.

P88
Microbial community gene expression of a cheese ecosystem through a metatranscriptomic analysis

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Introduction: Most of the studies dealing with cheese-ripening, although essential to monitor the development of product functionalities, have been global and descriptive in nature.

Very few studies deal with the mechanisms involved in the functioning of the cheese ecosystem as a whole. Using NGS tools, a new era in the characterization of complex microbial communities has emerged.

Objectives: We have investigated the functioning of a reduced cheese ecosystem. To do this, we combined metagenome and metatranscriptome analyses, together with microbial and biochemical data.The studied ecosystem has the advantage of being composed of well-identified, cultivable microorganisms whose genomes are fully sequenced and annotated. This ecosystem is composed of 9 microorganisms among others bacteria - *Lactococcus lactis, Arthrobacter arilaitensis, Brevibacterium aurantiacum, Staphylococcus equorum, Hafnia alvei, Corynebacterium casei* - and yeasts - *Geotrichum candidum, Debaryomyces hansenii, Kluyveromyces lactis*. In addition, this ecosystem possesses dynamic and functional properties which are reproducible and easy to quantify.

Materials and methods: A joint meta-transcriptomic, meta-genomic, microbial and biochemical approach has been undertaken at different times of the cheese-ripening process, and will be presented. Data analysis integrating quality control of sequences generated, statistical analysis of data, bioinformatics tools, has been developed.

Results: The identified cDNAs have been analysed (e.g. percentage of expressed genes of a given species in the whole ecosystem; percentage of expressed genes in each species, functional category classification of expressed genes) together with physiological data (e.g. microbial growth, aroma production, substrate consumption, proteolysis, lipolysis).

Conclusions: This integrated approach enables us to obtain an exhaustive and comprehensive view of the expression of a complex ecosystem. This gives a first picture of gene expression at the organism level within a complex ecosystem at work. Using this approach, the metabolic role of one given organism within a complex microbial community can be much better addressed. Such an approach could be applied for the functional study of other food ecosystems.

P89
Microbial characterization of drinking water in Lithuania

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Descriptions of water distribution systems microbial communities are actual for safe and suitable use as potable water. The goal of the study was to accurately characterize the microbial community structure within a drinking water distribution system in Lithuania using culture-based methods, biochemical techniques and a 16S rDNA phylogenetic approach. Clone libraries were developed and sequenced to more accurately characterize microbial populations in drinking water samples. Potable water (tap water, well water, borehole water) samples collected from different Lithuania places by membrane filtration were used to extract whole microbial community DNA. The commonest microbiological tests done on water were for coliform bacteria (*Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp.,), *Escherichia coli* (or fecal coliform), *Enterococci* (or fecal *Streptococcus*), *Pseudomonas aeruginosa* by an ISO standarts. A lot of non-fermentative gram - negative bacteria was found in centralised urban water. 16S ribosomal DNA from isolates of non - fermentative bacteria were amplified with universal primers. Studies showed that over 50% of the tested wells had an increased microbiological contamination by fecal colifrom bacteria and fecal *Streptococcus*. In centralised urban drinking water over 30% tested samples were detected *Pseudomonas* group, group NO-1 and others non-fermentative bacteria.

P90
Diversity and physiology of *Trichoderma* associated with *Burholderia* spp. in the nests of Borneo's exploding ants.

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Ants are often considered to be "keystone" arthropods in terrestrial ecosystems because of their high abundances and prominent functional roles in biological control of plant pests, habitat alteration, and nutrient recycling. Recent studies have shown that cavity nesting 'exploding ant' clade (*Camponotus* [*Colobopsis*] *cylindricus*, or COCY) has specific associations with such potentially beneficial nest microbes as species of a mycoparasitic filamentous fungus *Trichoderma* (teleomorph *Hypocrea*, Ascomycota) and nitrogen-fixing bacteria *Burkholderia*. Both microbes tended to co-occur in nest fiber, perhaps due to traits influencing their arrival and survival.
In this work we have studied the diversity and physiology of *Trichoderma* associated with COCY ants. We found that species detected from ant's nests fall into two ecological categories. The first group includes known highly opportunistic species cosmopolitan over many different ecosystems and geographic regions, which usually have high mycoparasitic potential. Second

group includes species which are likely specifically associated with COCY ants and or *Burkholderia* spp. bacteria. We tested the resistance of microbes to such volatile chemicals as m-cresol and diterpenes, which are secreted by COCY ants' mandibular glands. Cultivation independent (metagenomic) techniques were used to shed light on the main contribution(s) of both *Trichoderma* and *Burkholderia* to associated ants.

P91

Total microbial activity and microbial composition of a mangrove sediment are reduced by oil pollution at a site in the Arabian Gulf

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In a study carried out to determine the effect of oil pollution on the microbiota of sediment associated with mangrove in the United Arab Emirates, sediment samples were collected from oil-polluted and non-polluted mangrove sites. The levels of the total recoverable hydrocarbons and the polycyclic aromatic hydrocarbons assayed were noticeably higher in the polluted sediment. Microbial activity as measured by the hydrolysis of fluorescein diacetate and the total populations of the culturable aerobic and anaerobic bacteria, streptomycete and non-streptomycete actinomycetes, and filamentous fungi and yeasts were significantly (P<0.05) lower in the polluted than in the non-polluted sediment. The estimated total aerobic and anaerobic hydrocarbon-utilizing bacteria were significantly (P<0.05) higher in the polluted than in the non-polluted sediments. Four days after the addition of the water-soluble fractions of the light Arabian crude oil to the non-polluted sediment, at ten different concentrations, there was a significant (P<0.05) reduction (65%) in the microbial activity of the sediment compared to non-amended sediment. Concentrations of water-soluble fractions at 0.1% and above significantly and progressively reduced microbial activity with total cessation of activity recorded at levels > 50%. This study is the first to evaluate the effect of oil pollution on sediment aerobic and anaerobic microbial flora of mangrove communities.

P92

Mapping the niche of archaeal ammonia-oxidizers in Icelandic grassland soils - a field study and microcosm approach

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The contribution of ammonia-oxidizing bacteria and archaea (AOB and AOA, respectively) to the net oxidation of ammonia varies greatly between terrestrial environments. To better understand, predict and possibly manage terrestrial nitrogen turnover, we need to understand the process of ammonia oxidation and the ecophysiology of the associated organisms as a function of environmental conditions. We examined the independent and combined effects of mineral nitrogen deposition and geothermal heating on ammonia-oxidizing communities *in situ* by sampling soils from long-term fertilisation sites along a temperature gradient in Icelandic grasslands and by controlled microcosm studies in the lab. Microarray and quantitative PCR analyses of the ammonia monooxygenase subunit A (*amoA*) gene accompanied by physico-chemical measurements of the soil properties were conducted. Our field study showed that in contrast to most other terrestrial environments, the ammonia-oxidizing communities most likely consist almost exclusively of archaea, while their bacterial counterparts were undetected. Differences in distribution and structure of AOA communities were best explained by pH and clay content irrespective of soil temperature or fertilizer treatment in these grassland soils. However, it appears that AOB were not entirely absent. In a microcosm study we were able to demonstrate the appearance of the AOB population by incubation under elevated, but not under ambient ammonium concentrations. This evidently shows the strong competitive advantage of AOA over AOB at low ammonium concentrations. Our findings indicate that N availability may have a general potential for niche differentiation between bacterial and archaeal ammonia oxidizers, while differences in temperature, pH and clay contents may facilitate differentiation between AOA types in soil.

P93

Evidence for remarkable genetic plasticity of bacterial linear plasmids from extreme environments

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Members of the spore-less actinomycetous genus *Micrococcus* have a great potential for biodegradation and are, thus, of ecological importance for bioremediation. Moreover, they constitute a frequent source of biotechnologically relevant enzymes and bioactive compounds. *Micrococcus* bacteria withstand diverse harsh environmental conditions: they are able to survive in millions of years old amber samples, in carefully decontaminated space stations, and in oligotrophic, extremely saline and heavy-metal containing lakes of the high-altitude Argentinean Puna. Several strains isolated from the latter carry large, linear, extrachromosomal genetic elements with proteins covalently attached to their 5′ ends (termed linear plasmids). For a broader inspection of the potential of the linear plasmids for the survival and/or adaptation to such harsh environment, two different linear plasmids (pLMA1 and pLMA7, with sizes of 110 kb and 82 kb, respectively) were analysed in detail.

The plasmids were electro-eluted from PFGE gels and subsequently checked for blocked ends by exonuclease III and λ-exonuclease treatments. Sequencing was performed applying the Sanger technology in combination with 454 pyrosequencing. Remaining gaps were closed manually by PCR. Obtained sequences were analyzed using standard bioinformatics tools.

The elements were seen to share a rather conserved common backbone, consisting of 11 identically arranged sequence blocks comprising approximately 51 kb (G+C content 70.5 %), which are mainly composed of genes encoding essential plasmid functions and conjugal DNA-transfer. The plasmids differ in their respective accessory regions (G+C content on the average 66-67%), which consist of numerous mobile genetic elements potentially providing the basis for resistance to stress conditions and genetic plasticity.

The capability for conjugal transfer (facilitated by the common backbone) along with the multitude of transposable elements (present as accessory DNA) identifies the *Micrococcus* linear plasmids as extremely flexible, mobile, rather plastic genetic elements with the potential to enable the host bacteria to conquer and adapt to extreme ecological niches.

P94

Small-scale variation in bacterial community structure and function within freshwater ponds

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The extent to which bacterial communities exhibit small-scale biogeographic patterns in their distribution and function remains unclear, particularly within highly mixed freshwater environments. In this study, we investigate small-scale variability in bacterial community structure and function within a patchwork of shallow alpine tarns. Using a grid-based sampling design, we collected 100+ water samples located between 4 and 60 m apart in each pond. For every sample, variability in bacterial community structure was monitored using a DNA-fingerprinting methodology (ARISA) whereas differences in bacterial community function (i.e. carbon substrate utilisation patterns) were recorded from Biolog Ecoplates. The exact spatial location and dominant physico-chemical conditions (e.g., pH, water temperature, depth) were simultaneously recorded from every sample location.

Results of multivariate Mantel correlograms showed that, on average, bacterial community structure and function became significantly different comparing samples located 20 m or more apart. Variance partitioning revealed that purely spatial variation accounted for the more of the observed variability in both bacterial community structure and function (Range: 24-38% and 17-39%) than the combination of purely environmental variation and spatially structured environmental variation (Range: 17-32% and 15-20%). Contour plots of bacterial community similarity revealed greater spatial structuring in bacterial community structure than function suggesting that some of the changes in bacterial community structure are functionally 'redundant' (Fig 1).

Our investigation, which appears to be one of the smallest scale studies of bacterial biogeography conducted within lentic freshwater, reveals the presence of distinct bacterial communities across unexpectedly small spatial scales. We suggest that even within relatively mixed ponds, bacterial communities separated by distances of less than 20 m may be dispersal limited, differentiating at a rate which is faster than they are mixed together due to ecological drift. Our findings also suggest that most current sampling strategies collect samples at an inappropriate scale, overlooking significant spatially structured variability in freshwater bacterial community structure and function.

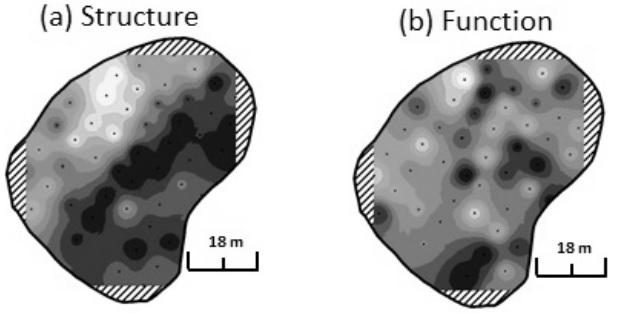

Figure 1. Similarity in bacterial community (a) structure and (b) function within a single tarn. Samples hosting more similar bacterial community data (as determined using a Bray Curtis similarity measure) are represented by more similar colours on each map. These plots reveal a stronger spatial pattern in bacterial community structure than function.

P95

How to make the most of agro-ecosystems – the impact of agricultural practice on microbial diversity and sulfur cycling

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Growth of healthy, high-yielding crop plants requires a stable input not only of nitrogen and phosphorus, but also of sulfur (S). In natural ecosystems, nutrient cycling is mainly mediated by soil microorganisms, and much research is devoted to optimization of microbial nutrient cycling for agricultural ecosystems. The diazotrophic plant-growth-promoting rhizobacteria *Azospirillum brasilense* and *Herbaspirillum seropedicae* are used as inoculation treatments for crops such as wheat, sugar-cane and rice in order to enhance nitrogen supply to the plant. However, these treatments do not enhance supply of other soil nutrients such as phosphorus (P) and sulfur (S), which mainly occur in plant-unavailable forms. Several rhizosphere microorganisms are able to mobilise plant-unavailable soil S, and two bacterial genes that may be involved in the process are *atsA,* which encodes arylsulfatase, and *ssuD* which encodes alkanesulfonate monooxygenase.

This study investigated the impact of agricultural practices on the overall rhizosphere microbial community and on functional diversity of S-mobilising organisms. Five wheat genotypes with different root-structure were inoculated with *A. brasilense* Sp7, *A. brasilense* Sp7-S or *A. brasilense* Sp245 to determine the influence of wheat genotype and inoculation treatment, in a continuous wheat field trial at Narrabri, New South Wales (Australia). Pot trials with vertisol soil from the field-site were carried out to investigate the effects of the different inoculation treatments and the effect of wheat variety under controlled conditions. NMDS-Analysis of fingerprinting profiles obtained by T-RFLP (Terminal restriction fragment length polymorphism) showed that wheat variety and inoculation treatment have a significant ($p < 0.05$) impact both on overall microbial diversity (16S), and on the *atsA* gene diversity in the rhizosphere. A comparison of two crop rotations, (field pea/sorghum/wheat or mustard/sorghum/wheat) also showed clear differences between the *atsA* microbial community in the two treatments. Ongoing work will help identify the key organisms that are responsible for these shifts in functional diversity, and whether changes in functional community lead to differences in plant-sulfur supply. Optimising plant-genotype and environment interactions by

taking into account the genetic potential of rhizosphere microorganisms can help to tailor more resource-efficient crop production systems.

P96

Characterization of *Agrobacterium tumefaciens* species complex isolates from agricultural soils of Slovenia

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Introduction: The causative agents of the crown gall disease are widespread soil bacteria of *Agrobacterium tumefaciens* species complex. Only some strains are causing tumors on a large variety of plants. The disease is economically important in fruit orchards in many countries, but not in Slovenia.

Objectives: Intrigued by low crown gall incidence in fruit orchards of Slovenia we explored the presence and distribution of *A. tumefaciens* in agricultural lands.

Materials & Methods: Agrobacteria were isolated from soil samples on selective medium 1A. Pathogenicity was tested on tomato, sunflower and *Kalanchoe daigremontiana*. Genomic species of *A. tumefaciens* species complex were determined based on *rec*A sequences (Costechareyre et al. 2010).

Results: The presence of *A. tumefaciens* species complex strains were detected in 63 of the 72 soil samples. All isolates were non-pathogenic and allocated into genomic species G1 (n = 43) and G4 (n = 26) (acc. nos. KC91939 - KC292007). Twelve new alleles were determined in G1 and five in G4. Alleles *rec*A-G4-7 and *rec*A-G1-10 were predominant. Different colony morphology between G1 and G4 isolates was observed on 1A and KB media (rough vs. smooth).

Conclusion: Presence of only non-pathogenic strains that we have detected in agricultural soils of Slovenia could (i) act as a natural protection against spread of pathogenic strains and the crown gall disease (New & Kerr 1972); or (ii) pose a risk for spread of Ti-plasmid (responsible for pathogenicity) among non-pathogenic strains if pathogenic strain would be introduced (Teyssier-Cuvelle et al. 1999).

P97

Is everything everywhere? – metagenomic analysis of Arctic and Antarctic marine bacteria communities

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The global ocean covers more than 70% of Earth's surface and has an average depth of ~3700 m, making it the largest ecosystem on the planet. Microorganisms inhabit every corner of the sea, from the deepest hydrothermal vent to the surface of the arctic oceans and comprise the largest biomass in the ocean. In the present work we investigate marine archaeal and bacterial communities from Arctic and Antarctic regions at three different depths (surface, medium and deep) to estimate the community composition and vertical and horizontal dispersion in the ocean. Next-generation sequencing is an excellent tool for investigating microbial diversity without the biases of cultivation dependent methods. Here we will present a phylotype analysis of 28 marine communities based on deep sequencing of 16S rRNA gene amplicons. The data indicates that the vertical distance (approx. 1000 m), rather than geographical location (horizontal distance - approx. 20.000 Km), directs the community composition, suggesting that very similar bacterial communities are occupying similar niches on opposite sides of the globe. Furthermore, the analysis reveals location specific phylotypes, which characterize different environments through the water column. These phylotype data are correlated with metagenomic data to further investigate the functions of the different environments.

P98

Do tree speices influence community structure and richness of ammonia oxidizing bacteria at three temperate forest sites?

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Introduction: The relationship between biodiversity and ecosystem function remains a controversial subject with numerous open questions. In Europe, the conversion of coniferous monocultures into broadleaved or mixed stand is considered to face ecological and economical risks posed by coniferous monocultures. Belowground effects of such a change in the dominant tree species is however largely unknown, although bacteria regulate many soil processes and some groups, like ammonia oxidizing bacteria (AOB) are highly sensitive to environmental stress.

Objectives: The aims of this study were to investigate (i) AOB community structure and richness under several tree species, (ii) microbial/environmental factors related to AOB diversity, (iii) the relationship between AOB diversity and the nitrification process.

Materials and methods: Forest floor (Of, Oh) was sampled under European beech, sessile oak, Norway spruce and Douglas fir at three sites. AOB community structure and richness was assessed by PCR-DGGE and sequencing. Samples were analysed for net N mineralization, potential nitrification, basal respiration, microbial biomass, microbial or metabolic quotient, pH, total nitrogen, extractable ammonium, organic matter content and exchangeable cations.

Results: AOB community structure and tree species effects on AOB diversity were site-specific. Factors regulating ammonium availability, i.e. net N mineralization or microbial biomass, were related to AOB community structure. AOB richness was not related to nitrification.

Conclusions: Our research revealed that, at larger spatial scales, site specific characteristics may be more important that tree species in determining AOB richness and community structure. Within sites, tree species influence AOB diversity. The absence of a relation between AOB richness and nitrification points to a possibly role of AOB abundance, phenotypic plasticity or the implication of ammonia oxidizing archaea in this process.

P99

Thaumarchaeota are pioneer organisms in acidic volcanic soils from South of Chile

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Nitrification involves the biological oxidation of ammonia to nitrate and is of fundamental importance in the global nitrogen cycle. Several studies have shown the relative contribution of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in different environments, but until now nitrification has not been examined in young volcanic soils. Volcanism in Chile is a continuous process that has a strong influence on landscape and geology. The aims of the present work were to evaluate the abundance and diversity of AOB, AOA and the total microbial community in acidic volcanic soils of different defined ages to determine their influence to nitrification and soil formation. Soil was collected from three vegetated sites recolonized after lava eruptions in 1640, 1751 and 1957 on Llaima Volcano, one of the largest and most active volcanoes in Chile. Quantitative PCR (qPCR), terminal restriction fragment length polymorphism (T-RFLP) and cloning of the *amoA* genes were performed for the AOA and AOB communities. The diversity of archaeal 16S rRNA genes was examined by cloning and sequencing and bacterial 16S rRNA by amplicon pyrosequencing. All soils showed high nitrification potentials, but were highest in the younger soils. Archaeal *amoA* genes outnumbered bacterial *amoA* genes at all sites and AOA abundances were found to be proportional to the nitrification potentials. Sequencing results indicated the presence of AOA related to *Nitrososphaera* and *Nitrosotalea*, and AOB related primarily to *Nitrosospira* and sporadically to *Nitrosomonas*. The study showed that both AOA and AOB are early colonizers of newly formed andisols, but that AOA outnumber AOB and play an important role in nitrification. In addition to confirming the importance of AOA in acidic environments with low ammonia concentrations, we have shown that AOA are the dominant ammonia oxidizers in these volcanic soils and colonize the soils early in their formation. We conclude that AOA are able to occupy potential niches that might not be available for AOB and can perform an important role in volcanic soil formation and function.

P100

Biogeography of soil *Burkholderia* populations

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The genus *Burkholderia* is an important component of soil microbial communities. *Burkholderia* species have a broad distribution in nature, occurring commonly in soil and in association with plants, fungi and animals, where mutualistic as well as parasitic interactions can be found. However, little is known about the factors influencing their abundance and diversity in natural environment such as soil. Literature suggests that pH could play an important factor in shaping the biogeography of *Burkholderia*. To assess this question, two geographical scale sampling sessions were conducted. The trans-continental scale sampling consisted of soils collected across North and South America, whereas soils collected from an agricultural field (UK) represented the local scale sampling. A quantitative PCR (qPCR) protocol targeting *Burkholderia* 16S rRNA gene was developed to analyse the relative abundance *Burkholderia* in the above-mentioned soil samples. Results suggest that pH had a significant effect on *Burkholderia* relative abundance in soils at both sampling scales: high relative abundance was observed in acidic and moderate acidic soils but in alkaline soils, *Burkholderia* were under the detective limit of our method. Furthermore, relative abundance was increased when soil was artificially acidified, suggesting that pH is a strong abiotic factor. The diversity of soil *Burkholderia* populations was analysed in a subset of 14 sites from the trans-continental scale sampling. 16S clone libraries were constructed for each site and a total of 675 sequences were obtained. Diversity analysis showed no correlation between pH and community composition, which was more influenced by factors linked to spatial distribution of samples and site elevation. The most abundant and widely distributed species was *B. glathei* which represented approximately 40% of all sequences. We are currently investigating the reasons underlying this preference of *Burkholderia* for acidic soils despite their ability to grow at neutral and alkaline pH in laboratory conditions. We hypothesize that biotic interactions, mainly related to the ability of *Burkholderia* to positively interact with soil fungi, are the basis of their geographic distribution and we are now investigating these interactions by using a proteomics approach.

P101

Effect of stratification on bacterial communities in forest soil and isolation of key bacterial taxa

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Coniferous forests represent a large biome in the boreal zones as well as a climax vegetation zone in the mountains of the northern temperate zone. They represent an important C sink and are thus of global importance. The main objective of the present study was to comparatively evaluate the bacterial community structure, in litter and humic horizons, from a *Picea abies* forest soil located in the highest altitudes of the Bohemian Forest mountain range and to isolate, identify and characterize the most abundant taxa with respect to their involvement in environmental processes. In order to obtain high accurate soil biodiversity results, three different DNA isolation methods were tested for both horizons previous to 454 barcoded pyrosequencing. Furthermore, five different solid media were used with the purpose of isolating the maximum number of different heterotrophic bacteria with a specific goal to enrich for slow growing taxa. Pyrosequencing results revealed that regardless of the strong acidic pH of soil, a highly diverse bacterial community was present in both horizons. Indeed, bacterial sequences belonged to 17 phyla which were recorded with abundances over 0.1%. *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were dominant in litter and humic horizons, comprising 74-88% of all sequences. Moreover, as in other low pH soils, *Acidobacteria* was the predominant phylum especially in the humic horizon, accounting for more than 40% of all sequences. More than 1100 bacterial were isolated from the forest soil belonging to 400 OTUs at a 97% similarity threshold. Among them, 45 OTUs corresponded to taxa recovered by pyrosequencing data and were subject to subsequent

physiological characterization. *Proteobacteria* was the predominant phylum among the isolated bacteria (62%) and also two different strains of *Acidobacteria* were obtained. This study confirms a strong bias among the analyses of bacterial community based on molecular approaches versus culture-based approaches.

P102

Impact of long-term mineral fertilization inputs on composition of bacterial, fungal and AOB communities and on microbial enzymatic activities

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Mineral fertilization of soil can affect activity and composition of soil microflora, however due to various environmental variables contradictory results have been reported on the effects of fertilization. In our previous study we showed that long-term mineral fertilization increased the proportion of *r*-strategists in soil, however it was not clear, whether the changes in the population of *r-K* strategists are linked to changes in the composition of microbial communities or by transition between the *r/K* states. Therefore, the aim of the present study was to investigate effects of long-term mineral fertilization on composition of bacterial, fungal and ammonia oxidizing bacteria (AOB) communities. Moreover, we tried to find out, whether the changes in community composition are also coupled with rates of selected enzymatic activities. Soils were sampled in 2009 and 2010 from a long-term experimental grassland field at Zavisin (Czech Republic) established in 1969 with completely randomized block design of five fertilization treatments: C (no fertilization), PK (32 kg P and 100 kg K ha^{-1} y^{-1}), 80N (80 kg N as NH_4NO_3 ha^{-1} y^{-1} with P and K), 160N (160 kg N as NH_4NO_3 ha^{-1} y^{-1} with P and K) and NF (320 kg N as NH_4NO_3 ha^{-1} y^{-1} with P and K, after 1990 no fertilization). Composition of bacterial, fungal and AOB communities was assessed by t-RFLP on bacterial 16S rRNA gene, fungal ITS region of rRNA gene and functional bacterial *amo*A gene, respectively. Selected enzyme activities were determined by fluorimetry using fluorogenic substrates. In contrary to bacterial communities, the fungal and AOB communities substantially differed between fertilization treatments, being especially different in the plots fertilized with N. The effects of fertilization were detected also on the NF plots with cessation of fertilization. The changes in community composition were coupled with decrease in activity of arylsulphatase and increase in activities of cellobiosidase, β-glucosidase, phosphatase and in nitrification activity. To conclude, composition of microbial communities is strongly influenced by long-term mineral fertilization, which is also mirrored in the microbial enzymatic activities.

P103

Drivers of ammonium oxidizing communities in soils from sugarcane fields in Brazil

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The oxidation of ammonia is the first step in the nitrification, typically carried out by λ- and β-Proteobacteria (AOB) and Thaumarchaeota (AOA). Here, we examine, in three units of sugarcane productions along the State of São Paulo, Brazil, the occurrence of spatially-shifts in abundance of such communities in relation to other factors, inherent to the cultivation of sugarcane that might be determinant in the size of such communities. In each area 10 sugarcane fields were sampled, where plants present contrasting characteristics of managements. Although not determined, the nitrification is indicated to be active in such soils by the prevalence of nitrate over ammonium in most of analyzed areas. Nitrate concentration was greater than ammonium in almost all soils (with 24,8 mg N/kg^{-1} dry soil), whereas only four of them showed ammonium in larger quantities. The quantification of targeted communities indicated that AOA community is more abundant than AOB in all analyzed areas. Numbers of archaeal *amo*A gene ranged surrounded the average of 10^6 copies per gram of soil, while this value for bacterial *amo*A gene was typically near to 10^3. Concerning intra-variations, nitrate concentration was higher in sandy soils (approximately 70%), possibly due to the predominance of aerobic system what favors the occurrence of ammonium oxidation. Bacterial *amo*A gene appears to correlate with soil pH, confirming its role on nitrification. Finally, these results implicate AOA as the primarily responsible for the nitrification process, and also indicate the physiological differences between AOA and AOB on soils with distinct textures. The subsequent analysis of our samples will generate further contributions to our knowledge of the global distribution patterns of AOA and AOB, and key driving factors for the assembling of such communities.

P104

Molecular analysis of microorganisms of deep subsurface thermal waters in Western Siberia

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Oil-bearing horizons represent extreme underground ecosystems characterized by anaerobic conditions, high temperature, salinity, and pressure. We analyzed the microbial community of the underground thermal waters (46-51°C) coming out of a 2775 meters deep oil-exploration well in Tomsk region, Western Siberia, Russia.

Two approaches were used to characterize the microbial community: identification of microorganisms based on amplification and pyrosequencing of variable fragments of 16S rRNA genes, and the sequencing of the total metagenomic DNA. The 16S rRNA analysis indicated the dominance of bacteria in community (81% of reads of the library), while hydrogenotrophic methanogenic archaea of the genus Methanothermobacter were accounted for the rest clones. On the other hand, metagenomic DNA sequencing revealed the presence of two dominant species in the community - Desulfovirgula thermocuniculi and Desulfotomaculum kuznetsovii. Other species were represented by the lineage close to the genus Thermoacetogenium and by uncultured Firmicutes. Contrary to the results of 16S rRNA analysis, methanogenic data showed that the level of archaea is low and accounted for only few percents. The results of metagenomic analysis suggest the following ecological relationships between microorganisms. Desulfovirgula thermocuniculi and Desulfotomaculum kuznetsovii are sulfate-reducing bacteria that can anaerobically oxidize low-molecular weight organic compounds and intermediate metabolites (lactate, butyrate, propionate) to acetate, and then to carbon dioxide. They are also able to grow autotrophically oxidizing hydrogene in

course of sulfate reduction. Bacteria of genus Thermacetogenium can hydrolyze organic polymeric compounds. Other non-sulfate-reducing Firmicutes, and possibly the organisms belonging to Ignavibacteriae, can also hydrolyze such compounds. Overall the results of this study provide new information regarding previously uncharacterized environment and show the value of high-throughput sequencing in the study of complex ecosystems. (This work was supported by the Ministry of education and science of Russian Federation (grant 14.132.21.1770))

P105

Microbial ecology of high-temperature springs of Caldera Uzon, Kamchatka

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The aim of our study was to analyze the microbial communities of hot springs located in the Caldera Uzon volcanic region (Kamchatka, Russia), which have different physical and chemical characteristics. The objects of the study were five acidic sources with different temperatures and two neutral hot springs ("Zavarzin" and "Burlyashii") with the temperature of 55-58°C and 90-94°C, respectively. Microbial community structures were revealed by pyrosequencing of 16S rNA gene fragments.

It was found that the microbial community of "Burlyashii" spring is dominated by only two groups of chemolithoautotroph microorganisms: Aquificales (69% of all bacteria) and Thermoproteales (91% of all archaea). The "Zavarzin" spring contains more diverse community, where thermophilic bacteria comprise an absolute majority. The level of archaea in this community is only about 5% of all microorganisms. The community contains both chemolithoautotrophic (aerobic and anaerobic) and photosynthetic microorganisms. The resulting organic matter may be fermented or completely oxidized due to the use of oxygen, sulfur or nitrate as an electron acceptor. "1884" source (pH 4.0, 50°C) is an artificial hole filled with ground water in the place where thermal waters moves hydrocarbons to the surface. This microbial community contains mostly archaea (more than 70% of all microorganisms), in majority affiliated with ''uncultured'' lineages.

Microbial communities of acidic hot springs, "1805" (pH 3.7), "1818" (pH 3.5), "1807" (ph 5.6), and "1810"(pH 4.1) also contain high fractions of archaea. Moreover, the majority of detected bacteria belong to mesophilic, but not to thermophilic microorganisms, and may originate from the surrounding low-temperature zones. The communities of acidic hot springs were dominated by two groups of thermoacidophilic crenarchaea, Sulfolobales and Acidilobales. It should be noted that significant quantities of some groups of archaea were found only in a few springs. For example, these are the representatives of Halobacteriales, Taumarchaeota and Nanoarchaeota found in some hot springs in high numbers. Overall this study provides novel insights into the composition of thermoacidophilic microbial communities and the nature of the ecological interactions among important taxa in these communities. (This work was supported by the Russian Foundation for Basic Research (grant 11-04-00671).

P106

Consequences of Amazon rainforest conversion: metagenomic analysis of soil-borne microbial community and their functional attributes

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The Amazon rainforest is the world's largest reservoir of plant and animal diversity, and it has been subjected to especially high rates of land use conversion by agriculture and pasture. Areas of native forest are cleared through slash-and-burn before being converted to their use. Here, we used shotgun metagenomic sequencing (>800 millions of sequences) to compare microbial communities composition and microbial functional attributes of 44 soil samples collected in forest, deforested area, soybean crop field, and pasture area, in the Brazilian Amazon region. The samples were collected in two years, during two sampling periods. The forest soil-borne communities were clearly taxonomic and functionally distinct from the altered soils and presented lower levels of alpha diversity. There was significant correlation between taxonomic and functional diversity, where higher taxonomic diversity is accompanied by higher functional diversity. The same pattern was observed for the two years sampling. *Proteobacteria*, *Acidobacteria* and *Verrucomicrobia* were abundant in forest soils. There was an enrichment of the phyla *Actinobacteria* and *Chloroflexi* in the deforested area, while soybean crop field and pasture presented higher abundance of *Bacteroidetes*, *Firmicutes* and *Planctomycetes*. The forest communities had higher relative abundances of genes associated with nutrient cycling, respiration, and photosynthesis. High abundance of cell division and cell cycle, metabolism of DNA, RNA and protein, were found in all altered soils. The soil used for agriculture and pasture presented an enrichment of sequences related to regulation and cell signaling, motility and chemotaxis, and cell wall and capsule. The network analysis revealed a more complex interaction between microbial and functional groups in forest samples, with lower interactions in pasture soils. These data indicate that the conversion of Amazon rainforest alters the microbial community structure and affects functional attributes by simplifying the community network after the land use change. Also, the metagenomics approach proved to be useful to increase our understanding of how microbial diversity and function vary across different land uses. *Support: FAPESP, CNPq and CAPES.*

P107

Effects of aromatic hydrocarbons addition in "Amazon Dark Earth" on the community and abundance of total bacteria and the catabolic gene *bph*

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"Amazon Dark Earth" (ADE) is a term used to describe horizons in Amazon soils. Due to high concentration of organic matter and black carbon, this soil became an important source to study genes

related to the degradation of aromatic compounds. Generally, catabolic genes are studied in aromatic compounds contaminated soils, so, this study aimed to examine the effects of hydrocarbons addition in ADE and their adjacent (ADJ) soils (no anthropogenic soils). Microcosms were constructed in order to follow changes in total bacterial community structure (16S rRNA gene) and aromatic hydrocarbon degrader bacterial community structure (*bph* gene) through fingerprint technique (T-RFLP), and also to analyze these genes abundance through qPCR, in soil samples before and after hydrocarbons incubation. It was used soil samples of four Amazon sites: two ADE soil sites, and two ADJ soil sites, both under different land use (secondary forest: FS; agricultural cultivation: CULT). The T-RFLP results showed no changes in total bacterial community structure after incubation for all studied soils. However, it was observed a shift in community structure of *bph* gene and a increasing of operational taxonomic units (OTUs), for this gene, after incubation for all studied soils. Principal Component Analysis of T-RFLP profiles of 16S rRNA and *bph* genes communities structure showed distinct groupings. In general it was observed clusters distinguishing ADE from ADJ soils (under FS and CULT) for 16S rRNA and *bph* genes. These clusters were observed for soils before and after treatment with hydrocarbons, which indicates that the structure of the studied genes was more influenced by the difference of anthropogenic soils in relation to their originals, than by the land use. The qPCR results reported a significant (Tukey, $p < 0.01$) increase in the number of copies of *bph* gene per gram of soil after hydrocarbons incubation for all soils. These results displayed the influence of aromatic compounds in the structure of aromatic hydrocarbon degrader bacterial community in ADE and ADJ soils. This study can shed some light on the role of catabolic genes in these soils and highlight the need to further study the factors controlling the diversity and function of aromatic compounds degrader bacteria groups in Amazonian soils.

P108

A long-term artificial soil study - minerals and charcoal control the microbial response to spiked litter and phenanthrene

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Minerals and charcoal were previously shown to be shaping factors of the microbial community composition. Since the subsequent effect of this interaction on the microbial functionality is poorly understood, we investigated the response of mineral- or charcoal-driven microbial communities in matured artificial soils to the effect of single or combined spiking of plant litter and phenanthrene (model pollutant). Artificial soil mixtures differed only in mineral content (montmorillonite, illite, ferrihydrite) or presence of charcoal and were incubated under constant environmental conditions with an identical aliquot of Luvisol microorganisms and sterile manure as nutrient source. After two years of maturation, phenanthrene (2 mg/g) was spiked to soils with/without plant litter (1 wt%) and incubated up to 63 days with a sampling on days 0, 7, 21, and 63. Regarding phenanthrene and litter response, microbial communities from artificial soils and from a similarly treated natural Luvisol were compared by 16S rRNA gene and ITS based analysis (denaturing gradient gel electrophoresis (DGGE), qPCR, pyrosequencing). Similar and mineral- or charcoal-dependent responses to phenanthrene between microbial communities from different artificial soils were observed by DGGE. Group-specific PCR-DGGE suggested *Actinobacteria* in artificial soils but *Betaproteobacteria* in Luvisol as strong responder to phenanthrene. Interestingly, litter addition, assumed to foster horizontal gene transfer, affected bacterial communities but did not increase the bacterial response to phenanthrene. In contrast, fungal communities responded only in presence of litter to phenanthrene.

Combined with chemical data, this study showed that the microbial response to litter and phenanthrene was influenced by the mineral- and charcoal-dependent microbial communities established and thus provides insights into the complex interaction network in soil.

P109

Distribution of degradation activities of woody materials in marine eukaryotes, thraustochytrids

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Objectives: Wood is composed of various chemical materials such as carbohydrates and lignin. In the mangrove area, it is very important to degrade these materials for nutrients cycle. This study was undertaken to investigate the distribution of woody materials-degradation activities in thraustochytrids known as important decomposers in marine ecosystem.

Materials and Methods: Thraustochytrids identified based on the 18S rRNA sequences were subjected to various agar plate assays. To evaluate the degradation activities of lignin, the agar medium containing Remazol Brilliant Blue R (RBBR) having similar structure with lignin was used. The degradation of RBBR was judged based on the disappearance of blue color around colonies grown on a plate medium. Bavendamm reaction was tested using a plate medium containing tannic acid and gallic acid. The reaction was detected by the color change to black or brown around colonies. To detect the cellulolytic activities, the CMC (carboxyl methylcellulose) agar pate medium was used. The plate was stained with 0.1% Congo red solution, and the production of cellulolytic activities was detected by appearance of clear zone around colonies.

Results: No degradation activities of RBBR were observed in all test strains. However, the blue pigment of RBBR was absorbed into the colonies of some strains. Bavendamm reaction was observed in genus *Aurantiochytrium* grown on the plate containing tannic acid. Extracellular cellulolytic activities were observed in strains except for genus *Aurantiochytrium*.

Conclusion: The results of plate assay indicated that thraustochytrids do not produce extracellular enzymes to degrade lignin, but they seem to absorb these materials into their cell bodies in some way. On the other hand, thraustochytrids except for genus *Aurantiochytrium* produce the extracellular cellulolytic enzymes. These result indicated that several thraustochytrids share the roles for the degradation of woody materials in the marine ecosystem.

P110

"High Resolution Melt" analysis: a novel method for fast screening of environmental samples for changes in bacterial phylogenetic composition prior to deep sequencing.

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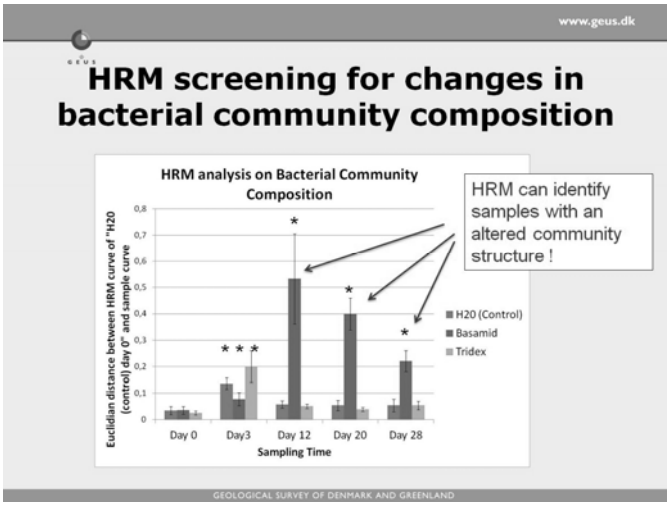
In the field of microbial ecology, most research projects result in a high number of samples. Although the price has gone down with the development of "next generation sequencing" it is often informative to screen your samples prior to deep sequencing. Traditionally this has been done with gel based techniques such as DGGE, which are slow, requires technical skill and give results that can be hard to compare between runs and laboratories.

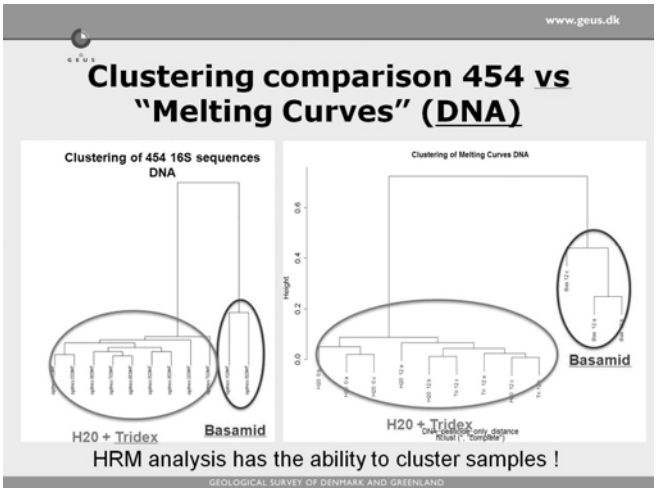
In this study we present a novel method for accurate and fast screening of phylogenetic composition of bacterial communities using "high resolution melt" analysis (HRM) on phylogenetic marker genes (e.g. 16SrRNA genes). Our intention for developing this technique is to produce a modern useful method for screening of large numbers of DNA and cDNA samples before deep sequencing.

Our test system consisted of 270 samples from soils which had undergone different pesticide treatments. First we amplified our phylogenetic marker gene (16S) using quantitative polymerase chain reaction (qPCR). This was followed by the production of a HRM analysis with precise measurements of relative fluorescence unit (RFU) with 0.1°C increments. The resulting curves were then normalized and the shapes of the individual curves were used as a measure of bacterial composition. In addition selected samples were also subjected to 454 amplicon sequencing as well as DGGE to confirm the findings of the HRM analysis.

The HRM results showed a large effect of one of the pesticides on the bacterial community structure, where as the other pesticide and the control showed no change during the experiment. These finding were confirmed with the data from the 454 sequencing and from the DGGE gel pictures.

In conclusion this novel screening method could be very valuable in identifying the phylogenetic composition of environmental samples early in the experimental setup, thus saving time and money on analyzing samples with no interest to the respective study.





P111

Effects of gentle remediation options (GRO) on the bacterial community structure of trace metal-contaminated soils

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Introduction: Soil contamination is a worldwide problem with serious associated environmental impacts and health risks. Gentle soil remediation options (GRO) are clean-up techniques based on the combined use of plants, amendments and associated microorganisms. GRO offer cost-effective *in situ* alternatives to conventional methods and potentially restore soil functions and quality. Phytoextraction removes trace metals from the soil through their uptake and accumulation by plants; whereas phytostabilisation aims to promote their *in situ* inactivation.

Objective: The aim of this study was to assess the bacterial community stucture and diversity several years after the implementation of GRO.

Materials and methods: Soil samples were collected from untreated and treated plots of four European field studies of the EU GREENLAND project (FP7-KBBE-266124) which have been managed for over 5 years with either aided-phytostabilisation or (aided)-phytoextraction (Biogeco (FR), Lommel (BE), Högbytorp site (SE), Freiberg-Halsbrucke (DE)). Community structure was studied using denaturing gradient gel electrophoresis (DGGE) focusing on the total Eubacterial community, *Alpha-* and *Beta-proteobacteria*, *Actinobacteria* and *Streptomycetaceae*. Similarities in DGGE fingerprints based on 16S rDNA amplified fragments were analysed using cluster analysis (UPGMA). Diversity indices were calculated: richness (S), Shannon-Wiener index (H') and Evenness (E). The influence of general soil physicochemical properties (pH, CEC, total C and N, available P), total (*aqua regia*) and phytoavailable concentrations of trace metals (H_2O-, NH_4NO_3-, $NaNO_3$- and EDTA-extractable) on the bacterial community were further assessed using canonical correspondence analysis (CANOCO v5).

Results: Clustering of DGGE profiles show clear differences in the structure of the bacterial communities between treated and untreated soils (at both the total community and group level). Few differences were found in diversity indices, but some increases were observed after GRO in the Biogeco site. At this site soils were amended with compost and dolomitic limestone or compost and zerovalent iron grit and planted with high-biomass plant species.

Conclusions: Application of GRO can lead to changes in the bacterial community structure and diversity.

P112
Microbial community composition in the traps and periphyton of two carnivorous *Utricularia* species

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Rootless, carnivorous *Utricularia* plants have been identified as prime candidates for future research on the complexities of plant physiology associated with carnivory and plant-microbe interactions. Previous studies mention the presence of diverse microorganisms inside the traps with a possible role in plant nutrient acqusition. A complex metagenomic survey of the trap communities has, however, been lacking. We have selected two representative species of aquatic *Utricularia*, *U. vulgaris* and *U. australis*, to asses the diversity of associated bacteria. Using NextGen sequencing (Illumina MiSeq platform) of 16S rDNA amplicons, we have surveyed the trap content as well as periphyton from plant tissues of different age. Traps of each species growing on different locations contained a significantly distinct community. The microbiome of *U. vulgaris* was dominated by Proteobacteria (53% of assigned sequences, p=0.013) while *U. australis* by Actinobacteia (45%, p=0.006). In contrast, there was significant overlap between the trap and the periphyton microflora on the same plant. Both plant species had similar protozoan and metazoan composition in the traps. A rich microbial community with distinct types of metabolism from aerobic methanotrophic Methylococcales to obligate fermenting anaerobes from class Clostridia was present even in the youngest traps of both species. The largely non-significant differences in microbial community composition between traps and periphyton indicate that, at least at the beginning of trap colonization, there is no strong selection for specific bacterial taxa, and that there is very small core group of bacteria associated with *Utricularia* traps. Some bacterial classes (Planctomycetia, Verrucomicrobiae), however, clearly benefit from the low oxygen and/or carbon-rich trap environment where they are significantly more abundant than in the plant periphyton.

P113
Endophytic bacterial communities from medicinal plants: a new source of bioactive compounds producing isolates

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Introduction: bacterial endophytes play important roles in plant biology, ad example promoting plant growth; they are also relevant in biotechnology as they produce bioactive molecules

with important medical applications such as in new antimicrobial compounds discovery. Besides, it is likely that the therapeutic properties of medicinal plants are also influenced by their endophytic communities as bacterial strains can directly produce bioactive compounds and/or elicit plant metabolism to produce them.

Objectives: this study aimed at the isolation and molecular characterization of bacterial communities from 3 relevant medicinal plants *Lavadula officinalis*, *Echinacea purpurea,* and *Echinacea angustifolia* to study the endophytes diversity dynamics (structure and composition) from the soil to the internal tissues of the same species and among species; we aimed then at the analyses of inhibitory capacity of endophytic isolates toward human pathogens and build a collection of bacterial isolates showing antimicrobial activity.

Material and methods: cultivable bacteria were isolated from surface sterilized tissues (stems, leaves and roots) and from the rhizosphere of the three medicinal plants; 16S rDNA sequencing and RAPD fingerprinting of 1000 randomly selected isolated were carried out, followed by taxonomic identification matching against RPD database. Antimicrobial activity testing was performed with the cross-streak method against human pathogens including Cystic Fibrosis opportunistic pathogens on the *Burkholderia cepacia* complex.

Results: i) a strong variation in the structure of the communities between the different plant species (although grown in physical proximity) and even in closely related species such as *E. purpurea and angustifolia* and in different organs/soil in the same species, with a low number of shared haplotypes, was registered; ii) a low overall diversity in the composition was nevertheless found; iii) many known PGPR species were identified; iv) several isolates have a strong antimicrobial activity against human opportunistic pathogens such as *Burkholderia* spp.

Conclusion: the results suggest a selection of the endophytic microflora by the plant species and tissue; medicinal plants appear to be an important source of interesting isolates with biotechnological applications potential.

P114

Pyrosequencing Analysis of enrichment culture used to remove perchlorate in spent regenerant brine

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Perchlorate (ClO4-) is an emerging contaminant of soil/groundwater and surface water. Perchlorate has been shown to block iodine uptake into the thyroid gland and cause a decrease in thyroid hormone production. Ion exchange (IX) is the most popular method to remove perchlorate from water due to its simple process, fast treatment time, and relatively low cost. Spent regenerant brine produced from regeneration of exhausted resin contains high concentration of salt and perchlorate and causes secondary contamination. Biological treatment of perchlorate is method of choice to degrade perchlorate in the spent brine since perchlorate-reducing bacteria (PRB) can convert perchlorate to harmless end products. In this study, ulfur-oxidizing salt-tolerant PRB were enriched in a fed-batch reactor that inoculated with activated sludge. The enrichment culture completely removed perchlorate in artificial spent brine (120 mg ClO4-/L and 50 g NaCl/L) as analysed by ion chromatography. PCR-DGGE and pyrosequencing were conducted to analyse microorganisms in the enrichment culture. DGGE analysis revealed that microbial profiles of the inoculum and enrichment culture were different from each other. Pyrosequencing result showed that 82.56% of the enrichment culture is *Thioalbus denitirificans* population. The enriched salt-tolerant PRB were used as inocula for the granular sulfur packed bioreactor that was used for the continuous removal of perchlorate in spent brine from IX. The result of reactor performance suggests that biological treatment of spent brine is environment-friendly and less expensive than disposal of spent brine.

P115

Impact of a genetic modification on bacterial diversity in the rhizosphere of maize across Europe

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The approval of genetically modified (GM) plants for cultivation in Europe requires a comprehensive assessment of their environmental risks. However, the effect of GM plants can be highly variable across contrasting biogeographic regions in Europe. AMIGA (*«**A**ssessing and **M**onitoring the **I**mpacts of **G**enetically modified plants on **A**gro-ecosystems»*) is an EU project studying possible environmental and economic impacts of the cultivation of GM plants that are relevant to European environments.

Soil fertility is one of the most important ecosystem services associated with plant production and largely influenced by soil microorganisms. Yet, how distinct rhizosphere microbial communities respond to a GM plant at different biogeographic conditions remains unclear.

Thus, within AMIGA we analyze the structural (16S rRNA gene) and functional (nirS and nirK as indicators for denitrification) diversity of rhizosphere bacteria of the GM maize MON810, producing the insecticidal *Bt*-toxin Cry1Ab against the European corn borer *Ostrinia nubilalis*. Rhizosphere soil samples of the GM maize and its near-isogenic, non-GM parental cultivar were collected from field trials in Slovakia, Spain and Sweden. *Archaea*, *Bacteria* and *Fungi* were quantified by real-time PCR and their abundance differed significantly for the three locations. Furthermore, genetic profiling of *Bacteria* by 16S rDNA T-RFLP analyses revealed significant differences in bacterial community composition among the three locations and between the GM and non-GM cultivar in Sweden and Spain, but not in Slovakia.

By ultra-deep sequencing, we expect to be able to distinguish a core bacterial community of maize from a more locally defined community which responds e.g. to soil properties, agricultural management practices or climatic conditions.

P116
Biodiversity of soil microorganisms in the Trindade Island/Brazil

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Introduction: Studies concerning oceanic islands have attracted scientific community interest, due to their unique characteristics, especially in regard to geographical isolation, relatively small size,and unique biodiversity. Prior research concerning soil microorganisms of oceanic islands are limited, reported only for high latitude islands closest to the continent, such as in the Antarctic Peninsula. It is well known that microorganisms play an essential role in terrestrial ecosystems, including soil fertility, residue decomposition and nutrient cycling, thus maintaining soil homeostasis. Despite its importance, the soil environment is considered to be one of the less well studied habitats and only recently have we began to understand that their biological and functional diversity is a crucial factor for maintaining all ecosystems.

Objectives: The aim of this work is to evaluate the biodiversity of microorganisms present in different soils on Trindade Island/Brazil, using state of the art molecular methodologies, together with more conventional soil analyses.

Materials & methods: Soil samples were collected at 12 sites on Trindade Island (The Brazilian oceanic island farthest from the continental coast). Genomic DNA was extracted and purified from each soil sample, and subjected to further analyses, including NGS (Next-Generation Sequencing) and T-RFLP on the bacterial, archaeal and fungal population, qPCR on the bacterial and fungal population, and more conventional soil analyses including physicochemical soil properties, microbial biomass carbon, etc.

Results: Preliminary findings show that samples differ regarding the number of phylogenetic markers (16S and 18S) gene copies, by qPCR. We obtained a total of 2,852,160 quality 16S Illumina® barcoded reads. Further analyses were performed following a closed-reference OTU picking protocol, using the QIIME toolkit. Principal Coordinate Analysis on bacteria 16S T-RFLP and Illumina® data were consistent, with replicates grouped together, and displaying similar outcomes. Further work includes data analysis of 18S Illumina® reads, ITS 454® pyrosequencing reads, and comparison of molecular data with physico-chemical soil properties.

Conclusion: This is a unique opportunity and the first effort in acquiring knowledge of the microbial diversity associated with soils of a Brazilian oceanic island.

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P117
Abundance and diversity of Clostridia in biogas production plants

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Biogas is produced in reactors under anaerobic conditions by a consortium of microorganisms which commonly include bacteria of the genus *Clostridium*. Since the genus *Clostridium* also harbors some highly pathogenic members in its phylogenetic Cluster I, e.g., *Clostridium botulinum*, there has been some concern that an unintended growth of such pathogens might occur during the fermentation process. A previous study revealed a selective effect of specific substrates used for biogas production on the bacterial and the clostridial community structures (Dohrmann et al., 2011). However, the knowledge on the dynamics and on the presence of bacteria with lower abundance is still very limited. Therefore this study aimed to quantify the populations of *Bacteria*, *Archaea* and of Clostridia Cluster XIVa and Cluster I by qPCR of their respective 16S rRNA genes. Cattle manure, pig manure or maize silage, each of three different origins, was fermented by a batch system and population sizes were followed over time. Independent of the applied substrate, target gene copies/ng DNA were 10^6 for *Bacteria* and 10^5 for *Archaea*. The contribution of Cluster XIVa gene copies of all *Bacteria* was 2-8% and that of Cluster I gene copies was 0.04-2.98%. The population sizes of all four target groups did not change over time, thus there was no indication of an increase in the proportion of clostridial populations. Additionally, samples from productive biogas plants were analyzed and the respective gene copy numbers matched those of the batch experiment. Currently, selective high-throughput sequencing of Cluster I clostridia 16S rDNA amplicions is used to identify clostridia present in the biogas plant.

References
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P118
Modeling of structure and Interactions the inorganic pyrophosphatase as from metagenomic sample

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Lately the natural diversity has been the best supplier for novel genes, enzymes and compounds in high requested by the biotechnological industry. In this sense, soils harbor an immense diversity of microorganisms, yet most remains unexplored. The metagenomic is a powerful method to study this search soil. Upon screening 10,000 clones, each one carrying recombinant cosmid exhibited inorganic pyrophosphatase (PPase, EC 3.6.1.1). The PPase is an essential enzyme catalyzing the cation-dependent interconversion of inorganic pyrophosphate and orthophosphate. This reaction provides a thermodynamic pull for many biosynthetic reactions. The recent application the PPase is an

additive in animal feeds and in the textile industry. The Bioinformatics is a step forward in proteomics of protein method appears as a strategy of choice to generate *in silico* hypotheses for experimental testing. The sequence of the protein 3D structure was predicted using the I-Tasser server. We can see PPase (A) protein structure prediction using the I-Tasser server the image (B) is a picture of the model constructed for your sequence based on that template. We considering that this is a new PPase as given below have been modelled with 100.0% confidence by the single highest scoring template is a true homology, code template PDB 4A01, i.d. was of 25%, Fold: H_PPase, Superfamily: H(+)-translocating pyrophosphatase. With these specials features on this PPase will perform cloning in vector pET28a; our results suggest that these characteristics obtained for this enzyme will further contribute to heterologous expression assays.

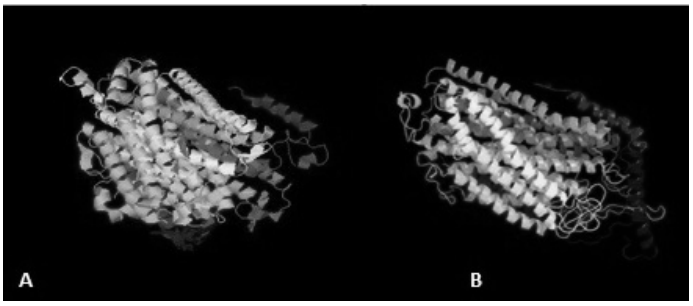

Figure 1: Constructing 3D models of our protein based on the alignments between the HMM of your sequence and the HMMs of known structure where A) PPase metagenomic and B) Template was code PDB 4A01.

P119
Reduction in bacterial functioning mirrors reduction in total and functional diversity after a strong soil disturbance

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Question: The relationship between biodiversity and ecosystem function has been extensively investigated. However, no consensus has been reached as to whether bacterial community diversity plays a role for ecosystem functioning.

Methods: We directly tested the diversity - function relationship by coupling functional measurements (degradation of arginine, a root exudate model compound) to diversity analyses of the functionally active bacteria. We set up communities with differing diversity through the application of short-time (3 hours) temperature stresses of increasing severity, followed by a one month recovery phase aimed at regrowth of the communities to similar biomass. Pyrosequencing of 16S rRNA genes fragment after the regrowth phase showed that the diversity in the stressed bacterial communities indeed gradually declined with increasing temperature stress, and that *Firmicutes* and *Proteobacteria* dominated in the stressed communities. Community function and diversity was then tested by spiking the different communities with a 13C-labeled arginine pulse (stable isotope probing-SIP). Functional diversity was analyzed by 16S rDNA pyrosequencing and T-RFLP of 13C-enriched DNA fractions of soil subsamples after 65 hours of arginine spiking.

Results: SIP results showed that the arginine degrader diversity declined in temperature-stressed soils, and a temperature stress of 80 and 100 degrees Celsius led to the appearance of new degraders, albeit from the same genus (*Burkholderia*). As test of community function, the kinetics of the O_2 consumption during arginine was determined and shown to be affected by temperature stresses. Curve fitting suggested that soils stressed at higher temperatures had lower initial concentrations of functionally active bacteria, representing the effects of changes in degrader biomass. Further, substrate respiration rates were faster at higher temperatures, pointing to the role of the presence of individual species.

Conclusion: These results document that functional diversity can determine functioning of substrate degradation, and that changes in overall community diversity were mirrored in a reduction in functional diversity.

P120
Ocurrence of bacterial laccase in Brazilian Podzols

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Podzols are soils of high occurrence in the coastal plains of the Sao Paulo State, and are characterized by the presence of a spodic horizon (Bh or Bhm). Podzolic soil formation involves the migration of organometallic complexes along the soil profile, which so called podsolization. Drainage can influence the process of podzolization and it is related mainly by the transport of organic matter through the soil horizons. Microorganisms play important roles in the genesis of Podzols, as previously observed in the Brazilian Podzols of Bertioga and Ilha Comprida. Well-drained podzols often show bleached mottles in deeper horizons, which are depleted in organic matter, probably due to the selective degradation of the soil organic matter by microorganisms. Laccases catalyze the oxidation of various substrates such as phenols, diamines and metals, coupled to the reduction of molecular oxygen. Laccases of fungal origin have been intensively studied but growing molecular evidence suggests that laccases may also be widespread in bacteria. Several potential substrates for laccases have been observed in Podzols of Bertioga, but there is no information on laccase activities and diversity in Brazilain Podzols. The aim of this study was to establish a quantitative-PCR protocol for the quantification of bacterial laccases in different horizons and mottles of Podzol profiles with distinct drainage regimes. Samples were taken from the characteristic horizons, and from bleached mottles and their immediate vicinity in the deeper horizons of three podzolic soil profiles, located at Ilha Comprida, Sao Paulo State, Brazil. Profile P12 was located at the sea front, and profiles 38 and 37 were located in a transect towards inland, at 50 and 100 meters from P12, respectively. Total DNA was extracted from soil samples and used for qPCR of bacterial laccases. Significant differences were observed laccase gene copy numbers among the profiles P12, P38 and P37 ($1.1x10^6$, $3.9x10^4$ and $4.7x10^5$ copies. g of $soil^{-1}$, respectively). Laccase gene copy number in bleached mottles was significantly higher than in their immediate vicinity only in P12 profiles. No significant differences were observed in P38 and P37. Higher abundance of laccase genes in P12 profile might be associated to the ocean influence on bacterial diversity as compared to the other profiles.

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P121

Subspecies diversity of the species *Novosphingobium acidiphilum* in Lake Grosse Fuchskuhle

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The *Alphaproteobacteria*-species *Novosphingobium acidiphilum* (freshwater tribe Novo-A1) represents a persisting phylotype of the epilimnic bacterioplankton in the acidic, humic acid rich south-west (SW) basin of the artificially divided Lake Grosse Fuchskuhle (NE Germany). Clone libraries (based on 16S rRNA gene sequences) of surface water samples revealed that 9% of the analyzed clones belonged to *N. acidiphilum*. During sunlight exposure, the SW basin of Lake Grosse Fuchskuhle generates substantial amounts of singlet oxygen, a highly toxic reactive oxygen species (ROS), due to the photosensitizer capacity of humic substances. Artificial increase in singlet oxygen enhanced *N. acidiphilum* abundance up to 66%. This strongly underlines the high adaptation potential of *N. acidiphilum* to humic acid rich, ROS generating, aquatic environments. The species *N. acidiphilum* was originally proposed based on a single strain isolated from surface water of the SW basin treated with singlet oxygen. Additional strains of this species (>99% 16S rRNA gene sequence similarity) could be isolated from the same habitat differing in pigmentation and growth. Genotypic differentiation, including *atpD* and AtpD sequence analysis, genotypic fingerprinting using ERIC, BOX, (GTG)5- and RAPD-PCR, combined with physiological differences in temperature- and pH-dependent growth experiments as well as singlet oxygen, hydrogen peroxide and UV stress experiments indicated the occurrence of three distinct *N. acidiphilum* ecotypes in the SW basin. The ecotype, which occurs at high abundance in the surface water during summer months, was highly resistant to singlet oxygen exposure but sensitive to hydrogen peroxide. In contrast, the two other ecotypes were adapted to increased hydrogen peroxide exposure - another ROS generated during illumination of humic substances. Both hydrogen peroxide resistant ecotypes were either isolated in summer after pre-enrichment with hydrogen peroxide or in winter after pre-enrichment with phenolic compounds. Our study shows that habitat-specific environmental factors have a high impact on the formation of ecotypes within the freshwater species *N. acidiphilum* in Lake Grosse Fuchskuhle.

P122

Seasonal effects on the bacterial community in upper horizons of soil profile in deciduous forest

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The bacterial communities living in forest soil contribute to the decomposition of organic matter and the recycling of nutrients in these ecosystems. However, the knowledge of the ecology of these communities is unclear due to fact that most bacterial species are not culturable. The study of the structure and composition of these communities may help to indicate which bacterial groups are involved in the decomposition processes, and how they respond to different environmental changes like the variation in nutrient supply due to seasonality. In this work, bacterial communities present in the upper horizons of a soil profile were studied along a seasonal cycle. Bacterial communities from litter (L), organic (H) and mineral (S) horizon were analyzed during the four season of the year by 16S rRNA gene pyrosequencing. Results showed that bacterial community composition, as measured by pairwise distances of phylogenetic composition, was horizon-specific. L horizon showed higher relative abundance of *Proteobacteria* and *Bacteroidetes*, while the H and S horizons presented a higher abundance of *Acidobacteria*, *Firmicutes* and *Actinobacteria*. In our study, the seasonal variations only affected the whole bacterial community composition in the S horizon. The community of S horizon was strongly affected by seasonality, showing a bacterial community during summer season being more similar to that present in the H horizon.

P124

Microbial communities in freshwater sediments – comparison of two metal contaminated sites by metagenomic approaches and resistance gene quantifications

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Background: Microbial communities have the potential to adapt in metal contaminated environments by developing specific resistance mechanisms. This resistance is mediated by different genes which are well known for some bacterial species. However, their abundance, their action and their diversity in metal-contaminated sediments is not well understood.

Objectives: The aim of this study was (1) to evaluate the quantity and the diversity of 3 resistance genes (*arsB*, *copA* and *czcA* for respectively As, Cu, Cd-Zn-Co) in sediments of 3 metal contaminated sites in the freshwater environment; (2) analyze the communities using shotgun metagenomics.

Methods: Degenerated PCR primers were designed for each resistance gene. Primer specificity and targeted gene diversity was assessed by metagenomics. Resistance gene quantifications were performed by absolute qPCR on DNA extracted from sediments. A specific protocol was designed to purify DNA before qPCR. Metal contamination was assessed in sediments by different approaches (total and sequential extractions, porewater analyses and DGT probes). For shotgun sequencing, DNA was purified and sequenced on a GS-FLX pyrosequencing platform. Sequences were then assembled and contigs were submitted to the MG-RAST pipeline.

Conclusions: Primer specificity was above 99% for the selected genes and the primer set targeted various genera of Proteobacteria (covering a large fraction of the studied bacterial population). Levels of *copA* and *czcA* were positively correlated to bioavailable Cu and Zn, respectively. These results suggest that the quantification of resistance genes could be a powerful tool to assess metal bioavailability in the environment. Shotgun

metagenomics indicate that many species are present as the abundance-rank curves obtained were almost flat. Burkholderiales and Pseudomonas are important bacteria in the sediments investigated, together with Leptothrix and Methylibium.

P125

Fungal-bacterial networks – an overseen potential to overcome limitations for effective contaminant degradation in vadose soils

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Introduction: Recent studies indicated the potential of hyphae as transport vectors for bacteria (*highways*) and contaminants (*pipelines*) in soil habitats. In addition to their degradation potential fungi may, thus, contribute to contaminant removal in soils by enhancing the limited bioavailability of the hydrophobic contaminants for bacteria. However, the ecological interplay of bacteria and fungi, working in physical and/or metabolic networks, was so far only marginally considered when contaminated soils were surveyed with respect to natural attenuation or biostimulation processes. Fungal-bacterial networks might represent a yet often overseen potential for the bioremediation of contaminated soils.

Objectives: Here, we aim at analyzing the ecological interaction of fungi and bacteria in contaminated soils by studying the community compositions of both microbial groups individually and with an integrative statistical modeling approach.

Methods: We surveyed a kerosene contaminated field site and analyzed the fungal and bacterial communities by molecular fingerprinting (T-RFLP and F-ARISA) in areas with different contaminant loads. In statistical analyses, we correlated the community compositions with environmental parameters (sampling site, depth, total contaminant load, concentration of 30 individual compounds) to identify the community-shaping factors.

Results: Bacteria were strongly responsive to contaminant quality and quantity. Communities in highly contaminated areas strongly diverged from communities at non-contaminated zones. In contrast, fungal communities were not influenced by any contaminant concentration. Their composition varied with the sampling site.

Conclusion: Bacteria were strongly impacted by the contaminant bioavailability whereas fungi appear to be more resistant against the chemical fluxes of hydrophobic pollutants probably attributed to the hyphal, multicellular growth form. Here, the distinct spatial location played a more important role for the community composition. Currently running modeling studies intend to reveal if bacteria and fungi work together in physical and metabolic networks at distinct environmental conditions or if they just co-occur, simply sharing similar niches and probably even competing for nutrients.

P126

Diversity of bacteria associated with natural vegetation growing in landfill of long-term PCB contaminated soil

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Introduction: Plants play some of the key roles in the soil bacterial diversity. It was proposed that in contaminated areas rhizosphere bacteria may help the plant overcome stress and toxicity associated with the contamination. Therefore, we sampled soil bacteria associated with plants growing naturally in the dumpsite of long-term PCB contaminated soil in Lhenice (Czech Republic).

Objectives: Our aim was (i) to investigate bacterial diversity in rhizospheres of 9 different plant species, one lichen and one moss; (ii) to study diversity of genes encoding biphenyl dioxygenases and benzoate dioxygenases present in the collected soil samples.

Material and Methods: In autumn 2012, rhizosphere soil samples of natural vegetation and a bulk soil sample were collected. From isolated rhizosphere metagenome, amplicons of bacterial 16S rRNA gene and genes encoding biphenyl and benzoate dioxygenases were prepared and pyrosequenced. The obtained data were processed with the Mothur software package.

Results: Proteobacteria dominated all studied samples although differences on the class level were observed. Considering functional genes, sequences of biphenyl and benzoate dioxygenases similar to known PCB degraders were found, together with some yet to be described sequences.

Conclusion: This project helps understand natural interactions between plants and bacteria in contaminated environment.

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P127

Symbiotic diazotrophic diversity and nitrogen fixation in the Brazilian Atlantic Forest

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The Atlantic Forest is a major "hotspot" of biodiversity and may be an important source of new microbial species and bioproducts. The process of biological nitrogen fixation (BNF) is the main form of nitrogen (N) input into an ecosystem and is mediated by several diazotrophic microorganisms. Among the diazotrophs, the legume nodulating bacteria are responsible for important fraction of the input of N in tropical forests. Information on the diversity

and activity of legume nodulanting bacteria in the Atlantic Forest is not known, but these diazotrophs may have an essential role in N-cycling and ecosystem sustainability. The aim of this study was to determine the occurrence and level of diversity of legume-nodulating diazotrophs and the rates of BNF in legume nodules along an altitudinal gradient in the Atlantic Forest. Root nodules were collected at four seasons, bacteria were isolated from the nodules and BNF rates estimated by the acetylene reduction assay (ARA). The analysis of genetic diversity was performed using partial sequencing of the 16S rRNA gene in addition to detecting the presence of the *nif*H gene. The data suggest a high diversity of nodulating nitrogen-fixing bacteria in the Atlantic Forest. Bacterial population showed no statistically significant differences when comparing low and high altitude sites, indicating that there is no selection of specific bacterial populations in the studied areas. In both areas, nodule associated isolates phylogenetically related to *Paenibacillus, Pseudomonas* and *Burkholderia* were most frequent, showing that these bacteria are important for legume nodulation and N input in the Atlantic Forest. Our results indicated a contribution of approximately 2.0 kg N ha^{-1} yr^{-1} due to BNF at low altitudes and 0.23 kg N ha^{-1} yr^{-1} at high altitudes.

P128

RNA and DNA-based profiling of soil microbial communities in conventional, innovative low-pesticide input and organic agricultural systems

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Introduction: New awareness of ecological and health impacts of pesticides has made the question of their use a central issue in agriculture. This trend has led the French Ministers for Agriculture and the Environment to propose a plan aimed at achieving a 50% reduction in pesticide use within ten years. Pesticide inputs can modify the microbial community composition and so affect microbial processes through changes in the soil structure or the availability of gas and nutrients.

Objective: The goal of this study was to observe how different pesticide inputs influence the microbial communities which are crucial for maintaining functioning of soil, and therefore soil health.

Methods: The total and active microbial soil communities (bacterial and fungal) were monitored in conventional (CV), innovative low-pesticide input (LP) and organic agricultural (OA) systems. These communities were investigated twice (March and July) over one cultural cycle by direct co-extraction of DNA and RNA from soils. Bacterial and fungal biomasses were quantified by qPCR targeting 16S and 18S rRNA from DNA and cDNA. Changes in total and active communities composition were analysed by T-RFLP analysis of bacterial 16S rRNA and fungal ITS.

Results: The analysis of physicochemical properties, agricultural managements and pesticide contaminations of each field allowed to check that the typology (CV/LP/OA) was the result of agricultural managements and pesticide contaminations, and not related to differences of physicochemical properties. The abundances of total and active microbial communities at the beginning of cultural cycle (March) showed any differences between the three management systems. The bacterial and fungal biomasses ranged respectively from 6.7 10^7 to 2.1 10^8 16S rRNA copies/g dry soil and from 8.1 10^5 to 5.5 10^6 18S rRNA copies/g dry soil. The active biomasses were 10 to 100 fold higher. The structure of microbial communities revealed diversity higher in total microorganisms than in active. On the contrary evenness was smaller in total communities. Analysis of TRF demonstrated that some were specific of field cultivated with conventional or organic managements.

Conclusion: The first results suggest that agricultural managements have an influence on total and active microbial communities but only in minority groups.

P129

Metagenomic insights into the rhizosphere of semiarid soils: functional conservation and phylogenetic variability in a drought mediated environment

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Question: The caatinga is the third largest biome of Brazil and is the only which is unique to this country. This biome has its characteristics molded by climate, the semi-arid, which is characterized by two distinct seasons, rainy and dry. However, researchers have neglected that biome for a long time, therefore not much is known about its ecological functioning and even less about the roles and lifestyle of its microbial inhabitants. Here, we presented an overview of the functioning and dynamics of the microbial communities found in rhizosphere samples from the caatinga with emphasis in comparing the effects of season in this communities.

Methods: With that purpose we applied metagenomic sequencing using ion torrent technology to gain an in depth view of those communities. The rhizosphere of the leguminous tree *Mimosa tenuiflora* was sampled in three different Caatinga sites during the peak of both seasons (dry and rainy) in a total of 6 metagenomic libraries. These were uploaded and annotated in MG-RAST server, and the annotation tables were used in several other analysis tools such as STAMP, Qiime and Metastats.

Results: We obtained 2.6 million reads with a total of 0.36 Gbp, The phylogenetic analysis indicated that phyla Actinobacteria and Proteobacteria make up to 50-90% of the sequences found in each library (fig. 1), followed by Firmicutes and Bacteroidetes, however, there was a large seasonal and spatial variation in the relative abundance of these phyla. Contrastingly, level 1 functional classification of this sequences has shown a great consistency within all samples (fig. 1). All major protein clusters found were related to basic metabolic functions. The application of Metastats test has shown that this pattern is indeed statistically significant (p

Conclusion: These results show that despite the the phylogenetic variation, there is a large degree of functional conservation which indicates that the metabolic profile must be important for life in the rhizosphere of these trees.

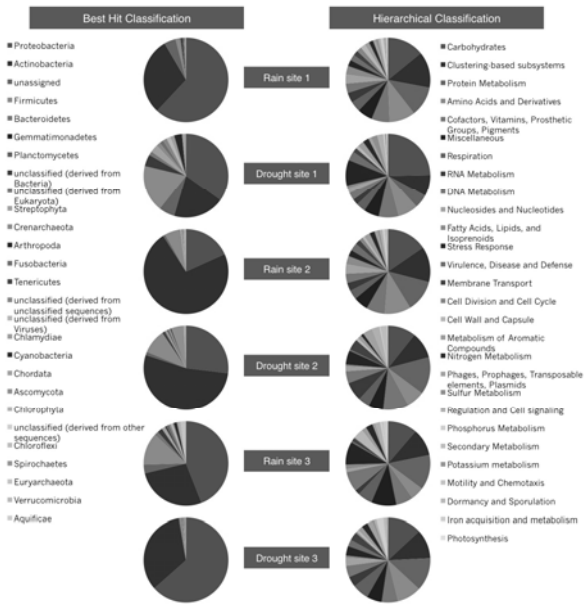

Figure 1: Community structure based on MG-RAST annotation.

P130

Quantifying cellulolytic bacteria in the rumen of sheep under a diet with sugarcane bagasse

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The microorganisms present in the rumen are active in the degradation of the plant biomass, and chemical processes involved in the breakdown of the cell walls are performed by different species. The success of micro-organisms in the enzymatic degradation of plant biomass provides subsidies for adaptation of the ruminants into several nutritional environments. Many studies have been conducted toward a full understanding of the host-microorganism relationships aiming the discovery of new enzymes to convert plant fiber into biofuels. In our study, we used real-time PCR to quantify the rumen cellulolytic bacteria *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* in sheep with or without sugarcane bagasse in its diets. Three animals were fed with a base diet and three animals were fed with a sugarcane-bagasse based diet for 60 days. Ruminal samples were taken over three time points (day 0, day 30, and day 60) and the total DNA was isolated for amplification and quantification of *F. succinogenes* and *R. flavefaciens* using the total 16S rRNA for normalization. This strategy enabled us to access the changes in these two bacterial taxa when sugarcane bagasse was introduced in the sheep diet. Both bacterial species were more abundant at the end of the evaluated period in the animals submitted to both diets. However, only *F. succinogenes* was significantly more abundant in the animals fed with sugarcane bagasse when compared to animals fed with the base diet (P≈0.0107). Our results showed that *F. succinogenes* responded to the presence of sugarcane bagasse in the diet, corroborating its role during the biomass degradation process. This will help us to focus in this bacterial group to explore enzymes to be applied in degradation of biomass material.

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P131

A bacterial ecology study to improve shelf life of food products: meat microflora in swine fed with polyphenols from olive mill waste

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Meat is an excellent substrate for bacterial growth and different factors (e.g. pH, redox potential, processing conditions) influence its microbial communities. Animal feeding strategy is the management factor most actively used as a quality control tool in meat production and the use of dietary antioxidants is recommended to preserve product quality. Olive mill wastes are sources of phenolic compounds with antioxidant and antimicrobial properties and a development of new bioremediation strategies is currently needed to overcome ecological problem.

This study is an evaluation of the employment of polyphenols obtained from olive mill wastes in the swine productive chain to identify possible correlation among feedstuff enrichment and meat microbial quality. To obtain a comprehensive view of the meat microbial biodiversity a 16S rRNA pyrosequencing approach was used.

RNA was extracted from nine meat samples conserved at 4°C, collected at 12 days from slaughter and derived from three groups of diets (control, phenolic extract and phenolic extract plus PUFA feedstuff enrichment). cDNA was amplified with primers targeting the V3-V4 region of 16S rRNA gene and analyzed using the 454/Roche GS Junior technology. Subsequent analysis were carried out using the QIIME (*Quantitative Insights Into Microbial Ecology)* pipeline.

Dietary enrichment resulted in a increased microbial α-diversity and richness. The enriched diets significantly decreased the relative abundances of *Pseudomonas* and *Brochothrix* genera. In particular, *Pseudomonas* spp. constituted 83.9% of OTUs in control samples and 38,6% in meat derived from animals fed with phenolic extract plus PUFA. These interesting effects are accompanied by a increased abundance of the *Lactobacillales* order in the treated samples.

Phenolic extract clearly influences the composition of the swine meat microflora and seems to ameliorate flesh quality and shelf-life.

P132

Spatial and seasonal variation of the rhizospheric bacterial community of two legumes of Brazilian semiarid uncovered by large-scale sequencing by Ion Torrent

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Question: Dry environments are present and well distributed on all continents. This characteristic can be presented in full-time or seasonal period (arid / semi-arid). In this context, it is found the Caatinga, a biome of Brazil classified as semi-arid. Legumes are quite abundant in this region, many of which are endemic. Therefore, This study aims to characterize the bacterial community present in the rhizosphere of two plants (*Mimosa tenuiflora* and *Piptadenia stipulacea* (*Benth.*) Ducke) representatives of the family Leguminosae, in order to assess whether there is variation in the composition of this community compared to seasonal, geographic and plant species factors.

Methods: Therefore, rhizospheric material was collected from Bahia, Piauí, Ceará, Paraíba e Rio Grande do Norte states. The rhizospheric soil was sampled from two plant individuals in two different seasons, summer and winter (dry, rain, respectively), the region V6 16S of rRNA bacteria present in this soil was amplified and sequenced through the sequencer Ion Torrent and the data was assessed in the software Mothur.

Results: It was found a significant variation when comparing the wet and dry seasons (Figure 1); in contrast, no change was observed in relation to plant species or geographical location, except the state of Bahia, which has gathered over the other regions. The most representative phyla in both plants were of Proteobacteria, Actinobacteria and Acidobacteria; the variation in relative abundance with respect to seasonality occurred in all of them, with increased population of Proteobacteria and diminution of the other two in the rainy period, situation which was also observed for both plants (Figure 2). In an RDA analysis, forward selection with a Monte Carlo test shows that Zn (p = 0.002) and SCC (p = 0.008) are the characteristics that shape the community having an effect on the phyla Firmicutes and Proteobacteria.

Conclusions: The bacterial community structure is profundly shaped by drought with the decrease of proteobacteria during this season.

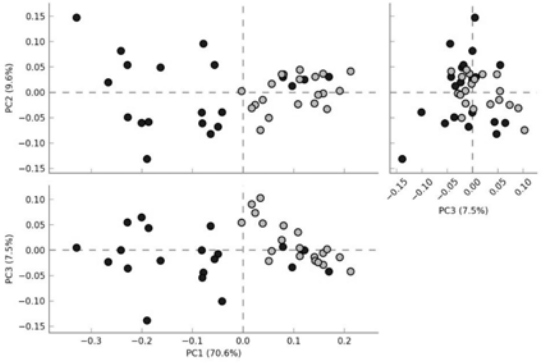

Figure 1: PCA of the sequences classified by the seasons (wet and dry) using STAMP.

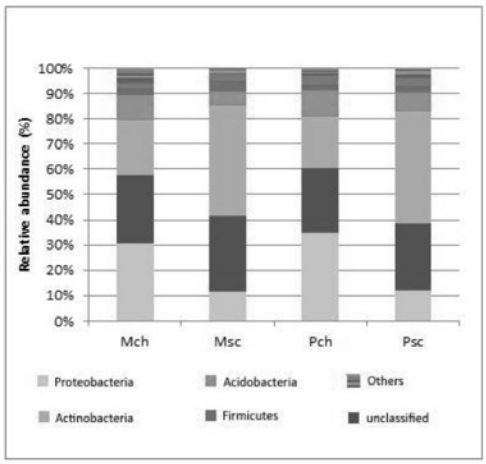

Figure 2: Relative abundance of the most representative phyla at the different collection points in the dry and rainy seasons.

P133

Bacterial diversity of sugarcane cultivated soil comparing V_3 region from 16S rRNA

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Introduction: The diversity of microbial communities is used as a soil quality indicator, since they respond quickly to sudden environmental changes. The soil utilization processes, such as crop production, mainly on monoculture areas, might cause significant losses on the microorganism diversity, with different side effects on the environment as well as to crop production.

Objective: To study the bacterial diversity of an area of intense cultivation of sugarcane, using the fragment V_3 of the gene 16S rRNA and to compare such population with that of a forest area was the objective of this work.

Material & Methods: Metagenomic DNA samples were obtained from soil types and used for the amplification of this fragment . Fragments of DNA sequences were obtained using Illumina's sequencing platform. These sequences were analyzed by comparison of them with those of the Ribossomal Databse Project II (RDP II).

Results: It was observed that the phylum Proteobacteria was the predominant among all samples (Native Forest 44% and Sugarcane 52%). The phyla Acidobacteria, Actinobacteria and Firmicutes presented roughly 2% occurrence at the native forest areas, although at the sugarcane areas the mentioned proportions changed to 1.5%, 3% and 4%, respectively. Besides these ratios, representing only 1% on the sugarcane samples and 2% for the native forest areas, it was observed members of the following phyla: Armatimonadetes, Chlorofexi, Gemmatimonadetes, Nitrospirae and Planctomycetes. Nutrient composition and humidity changes that regularly take place during the crop production cycles, considering several monoculture areas, including that of sugarcane might be the key factor for the presence of greater amount of members of the Firmicutes phylum

at that particular cultivated area. This might represent a development strategy by these species, that takes place in these crop adapted regions, to explain the fast growth rates observed, when nutrients are available and the formation of spores when the opposite situation takes place, the lack of nutrients and humidity low levels.

Conclusion: Production of sugarcane determines the modifications on soil bacterial populations, such that they cause effects on the recycling the soil chemical elements, influencing the production of greenhouse gases, reducing the levels of available soil carbon and causing the acceleration of global climate changes.

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P134

Soil quality and microbial community changes after a decade of different tillage at two Slovenian sites with different pedo-climatic conditions

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Conservation tillage systems are recognized as important strategy to maintain/increase soil quality and carbon sequestration; however the complex biological, chemical, and physical interactions are site and management specific. The effects of reduced tillage (RT) in comparison to conventional ploughing (CT) on soil physical and chemical properties and consequently on microbial community structure were evaluated in a long-term field experiments at two different locations. Site Moskanjci is characterized by sandy loam texture (Eutric Fluvisol on gravel and sand), precipitation 991 mm/a, mean annual T 10.7 °C; at site Ljubljana soil is silty clay loam (Eutric Gleysoil), precipitation 1400 mm, mean annual T 10.9 °C. Soil samples were taken within the top two layers (0-10 cm and 10-20 cm) in November 2011 after 12 years of experiment duration. RT system had positive effects on soil organic carbon in the upper 10 cm at both sites (1.60 % ±0.07 and 1.45 % ±0.05 at Moskanjci; 2.88 %±0.19 and 2.38 %±0.11 at Ljubljana), as well as on nutrient contents (N, P and K), soil aggregate stability, and water holding capacity. Water infiltration rate was also higher in RT. Microbial biomass estimated by the total soil DNA content was significantly higher in RT than CT soil in the upper 0-10 cm soil layer at both locations (13.2 and 10.0 µg cDNA/g d.m. soil in Moškanjci; 18.79 and 14.03 µg cDNA/g d.m. soil in Ljubljana). Microbial community structure estimated by terminal restriction fragment length polymorphism (T-RFLP) fingerprinting method of archaeal 16S rRNA genes, bacterial 16S rRNA genes and fungal ITS regions, showed that fungal communities in the upper 0-10 cm soil layer in Moskanjci were affected by the tillage system, while in Ljubljana RT and CT in the topsoil showed no differences, which can be explained by the crop rotation (alfalfa on Ljubljana site grown for the last two years). Bacterial community had not been affected by the tillage in the upper 10 cm. However, fungal and bacterial community structures were influenced by the soil depth in RT at both sites.

P135

Culturable actinobacterial diversity from a Brazilian restored forest fragment

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Most actinobacteria species is still unknown and in different ecological niches our knowledge about the ecology of these microorganisms is nonexistent. Furthermore, 50% of the known antibiotics are produced by actinomycetes of which a single genus, *Streptomyces*, is responsible for 90% of these compounds. Nevertheless, other genera have been demonstrated as good producers of novel classes of antibiotics. Therefore, this economical and biotechnological appeal has created high expectations on the discovery of new species of actinomycetes and the possible compounds they may produce. Brazil has been recognized as one of the largest areas of biodiversity in the world and "Mata Atlântica" has a considerable participation in this title. Many microbiological studies have been realized in native areas of this forest, however, only few works have focused on identification of bacterial species in restored areas. Therefore, the main aim was characterize the actinobacteria diversity from soil of a forest fragment in early (10 years) process of restoration, located in the state of São Paulo. Six different isolation media were used. A total of 84 bacterial strains were isolated and clustering into 36 morphotypes prior to characterization by 16S rRNA gene sequencing. The SM3 medium was the most suitable to access different actinomycete genera (n=4). The 16S rRNA gene analysis demonstrated high actinomycete diversity, with 25 *Streptomyces* spp. (the most predominant morphotype) followed by six morphotypes of *Amycolatopsis* spp.. The phylogenetic analysis showed close affinity between the isolated morphotypes and free-living actinomycetes. Our data corroborated with some uncultured-based approaches in demonstrating a vast diversity of actinomycete in soils from restored forest fragment. However, once the culturable actinomycetes have been identified, further studies could now focus on the biotechnological exploration of this isolates.

P136

Structure of bacterial communities of *Vigna unguiculata* (L.) Walp. rhizosphere soils in dark soil from amazon

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Bacterial communities of dark soils at Amazon (ADE) feature a wide variety of genes that are still unknown and may be important for biotechnology and sustainable agriculture practices. Currently, it is of great interest the knowledge of rhizosphere bacterial communities due to their key role in this ecosystem in association with the metabolism of the host plant. This study aimed to evaluate the structure of the bacterial communities in the rhizosphere soils of cowpea [*Vigna unguiculata* (L.) Walp] and compare them with non-rhizosphere dark soils and their adjacent (ADJ) by using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. T-RFLP data were analyzed through files generated by Data Collection program and analyzed with Peak Scanner 1.0 (Applied Biosystems) to determine the length of the terminal restriction fragments (T-RFs). MDS

(Multidimensional Scaling) profiles of T-RFs were generated for three different environments. T-RFLP data were correlated with chemical attributes of soils and were sorted using Redundancy Analysis (RDA). Spatial ordinations were set for the bacterial community combined with soil attributes. The two-dimensional representation of MDS analysis was validated by 0.04 Stress levels. Our results showed groups to the similarity level of 55% among samples and therefore high similarity among replicas of the samples. RDA analyzes of T-RFLP profiles showed that these bacterial communities differ in structure and these differences are directly related to the chemical properties of soils, as these accounted for 74% and 7.2% of the total variability of data, respectively. Among the attributes, P and Al were the elements most associated to the variability detected. Our results revealed that the structures of the bacterial communities in the rhizosphere of cowpea beans presented significant differences when compared with soils with TPA and the ADJ. These results demonstrate that the interaction of plants and soil microorganisms contribute to shape the microbial soil communities, which act in the rhizosphere of cowpea plants. These results highlight the possibility of characterization these contrasting bacterial communities by genomic sequencing for taxonomical and functional diversity.

P137

Functional microbial community shifts due to bacterial invasion revealed by community level physiological profiling in a soil dilution-to-extinction experiment

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Any species that is introduced into a foreign range, manages to survive for an extended period of time, and impacts the local community is an invasive species. Reports of microbial invasions are growing, and one ecosystem under study is soil. *Escherichia coli* introduction and subsequent survival is a prime example of a microbial invasion. Recent research has shown that the diversity of the native microbial community is a large explanatory factor of pathogen survival in soil. Two further questions are how diversity actually functions, its mechanisms *per se*, and how invasion can impact the microbial community. In a dilution-to-extinction experiment, we used two types of natural soils (B and W) to create dilution series where the 10^{-1}, 10^{-3}, and 10^{-6} dilutions from each community were separately inoculated into sterile soil (W) and incubated to reach similar microbial abundance. The invader *E.coli* O157:H7 was then introduced, and its survival was followed over 75 days by plating on selective media. Community level physiological profiles (CLPP) were obtained both before and 28 days after invasion of communities in order to understand how the introduced communities used carbon sources and how invasion would influence their CLPP. Dilution treatments did significantly affect *E.coli* survival 28 days after invasion (P<0.015), and both the speed and quantity of resource use helped explain the pathogen's fate (R^2=0.35-0.62; P<0.005). CLPPs were significantly different in non-invaded and invaded communities (P< 0.0002). Furthermore, while community and invader profiles overlapped before invasion, a large amount of variation was seen after invasion. This may indicate the community has undergone selection for species that utilize resources other than the invader's and/or that the resources released due to invader death has promoted a shift in community structure. Further analyses of bacterial community structure are needed to validate these hypotheses. Overall, we show support that microbial diversity, as a result of dilution treatments, influences the potential of a community to extract carbon from its environment, and this potential well explained the survival of *E.coli* in soil microcosms. We also show that invasion is a large perturbation to a microbial community, with the potential to change community structure.

P138

Combination of stable isotope probing and metatranscriptomics to examine the activity of aerobic methanotrophs in lake sediment

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Little is known about the functioning of microbial populations in natural environments. Stable isotope probing (SIP) and metatranscriptomics are two approaches that can be used to identify active groups of microorganisms and to investigate their behavior. The objective of this study was to combine these two methods to first isolate the mRNA of the active methanotrophic bacteria in lake sediment by SIP and obtain a targeted metatranscriptome of the labeled RNA. The methanotrophs in lake sediment were labeled using $^{13}CH_4$ and both labeled and unlabeled RNA were isolated and sequenced by 454 pyrosequencing. The unlabeled metatranscriptome had a large diversity of bacterial, archaeal, eukaryotic and viral sequences as expected from a diverse sediment community. In contrast, the labeled-RNA metatranscriptome was dominated by methanotroph sequences, particularly from *Methylococcaceae*. Transcripts of the methane monooxygenase genes *pmoCAB* were the most abundant in this metatranscriptome and the pathway of methane oxidation to CO_2 could be traced as well as many steps in the ribulose monophosphate pathway for carbon assimilation. A high abundance of several different transcripts for chemotaxis, motility and attachment proteins were detected, suggesting an importance for methanotrophs in lake sediments. This approach should be broadly applicable to SIP experiments and will enhance the detection and identification of mRNA from target organisms and enable unique insights into the physiology and function of the target microorganisms living within complex communities.

P139

Abundance and community structure responses of sulphur oxidizing bacteria during elemental sulphur oxidation in soil microcosms

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Sulphur oxidising bacteria (SOB) play a key role in the biogeochemical cycling of sulphur (S) in soil ecosystems. As yet, the ecology of these bacteria is poorly understood, and factors affecting S-oxidation have not been defined. We have developed a molecular approach to study abundance and diversity of SOB in soils based on analysis of the soxB gene encoding the soxB component of the Sox enzyme complex, widely distributed among

several sulphur oxidizing bacterial taxa. Primers specific to the soxB gene of SOB relevant to soil systems were designed that allow estimation of both abundance (based on real time PCR assays) and diversity (based on cloning and sequencing). Results demonstrate links between abundance of soxB gene and sulphur oxidation as estimated by sulphate production measurements in soil microcosms amended with elemental sulphur. Cloning and sequencing revealed considerable diversity of soxB genes from New Zealand soils. Clone sequences were affiliated with soxB gene sequences of several clusters within alpha- and beta-proteobacteria of a variety of known auto- and hetero-trophic sulphur oxidizers. Elemental S application had a significant effect in the soxB gene diversity with the chemolithotrophic Thiobacillus-like betaproteobacteria sequences dominating the clone libraries 6 days after sulphur application although this group was undetectable in the start of the experiment. This study provides evidence for links between SOB abundance, diversity and function and the methodology provides a new tool to investigate the ecology of SOB, allowing for better understanding of soil S biogeochemistry.

P140
Efficiency of three extraction methods of PCR amplifiable DNA from protozoa

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Diversity and function of soil microorganisms has been evaluated based on total DNA extraction from environmental samples. DNA extraction from a variety of biological samples is a routine step in the field of molecular genetics. In this context, significant efforts have been devoted to optimize soil DNA extraction procedures to obtain representative extracts for quantitative and qualitative characterization of microbial communities. Soil eukaryotic microbes, especially protozoa lag behind other microbes despite their rather important contributions in soil ecosystem services like, carbon and nitrogen cycles.

The aim of this work is to find the most optimal technique of extracting protozoan DNA from soil. The efficiency of three different DNA extraction methods for protozoan populations and from soil samples was evaluated. The techniques compared were a modified version of ISO-11063 method (Plassart et al., 2012), the GnS-GII protocol (protocol developed by GenoSol platform to extract soil DNA in large-scale soil surveys), and a commercial DNA extraction kit (MoBIO). Pure cultures of different protozoan strains (amoebae, ciliates and flagellates) and environmental soil samples including a wider range of physicochemical characteristics were used to test the different extraction methods. Different strain specific and general protozoan primers were used to test the efficiency of the amplifiable DNA. The DNA yield and purity, success rate of PCR amplification as well as the denaturing gradient gel electrophoresis (DGGE) profiles were analysed. Through this analysis we will be able to improve future investigations of communities of soil protozoa and their impact on soil ecosystem functioning.

P141
Exploring glyphosate-induced effects on turnover of root biomass and on rhizosphere microbial community dynamics in plant-soil mesocosms

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Soil is a very complex and species-rich habitat which provides important ecosystem services beneficial for humankind. Bacteria and protozoa are key organisms for biomass turnover in soil and play an important role in plant nutrient availability and soil quality. In Denmark, glyphosate is used extensively as a non-selective herbicide and for ripening in plant production. Due to accelerated changes in root death and degradation this use may affect biogeochemical cycles and soil communities. The main objectives are to study the link between microbial communities in soil rhizosphere with variable carbon availability (Glyphosate treated vs untreated) and to get insight in how bacterial-protozoan interactions and biodiversity influence carbon cycling. Microbial community dynamics are studied in barley (Hordeum sp.) rhizosphere after glyphosate treatment in mesocosms. Total organic carbon availability is analysed (TOC) and effects on the soil microbial communities are assayed by genetic fingerprinting (DGGE) and quantification methods (CFU of bacteria, MPN of protozoa) along time. Results assess the effect of increased carbon availability due to glyphosate treatment on respiration, soil microbial community interactions, abundance and diversity. Moreover, the potential of the selected techniques as reliable indicators of specific soil functions is evaluated. A deeper knowledge about glyphosate-mediated effects will permit us to predict environmental changes, protect resources and services and obtain those in a more sustainable way.

P142
Microbial diversity and functional annotation of sugar-cane cultivated soil by next-generation sequencing approach

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Introduction: Sugar-cane is currently the most important crop of the São Paulo State (Brazil), whereas the agroclimatic zoning studies indicate at least two environments with distinct cultivation conditions. Nowadays, due the expansion of the agricultural borders, is highly desirable the increase of a sustained productivity of the sugar-cane cultivars using the knowledge of microbial soil diversity. The new sequencing technologies approaches have become a very powerful tool for microbiological taxonomical and functional studies in environmental samples.

Objectives: Therefore, the main goal of this study is related to survey the taxonomic distribution and functional classification of the microrganism present in sugar-cane cultivate soils from Sao Paulo State (Brazil).

Materials & Methods: The metagenomic DNA concentration where estimated using Qubit (Invitrogen). The genomic libraries were constructed using the paired-end protocol (2x100bp) and sequenced using the Illumina® HiScanSQ platform. The bcl files were converted to fastq file using the CASAVA 1.8.3, and further analyzed by the CLC Genomics Worbench 6.0.1 software.

Results: There were obtained 60,202,952 reads, whereas 15,549,68 reads were grouped in 580,515 contigs, and 44,652,984 reads were attributed as singlet read. All reads were submitted to MG-RAST platform for taxonomic classification and functional annotation. The majority of the reads (92,4%) were classified in bacteria domain. The most representative phylum are: *Proteobacteria* (40,4%), *Actinobacteria* (12,8%), *Acidobacteria* (8%), *Firmicutes* (6,6%), *Verrucomicrobia* (5,5%) e *Bacteroidetes* (3,9%). Functional predictions indicate that at least, 63,7% of the reads were classified as protein with unknown function, and in only 36.3%, reads were determined a putative function. Among the functionally classified reads, 39,2% are related to metabolism process, 21.5% to environmental information processing, 16.7% to genetic information processing, 11.2% to cellular process, 6.5% to human diseases, and 4.9% to organismal systems.

Conclusion: These results corroborate previous studies that indicate a high prevalence of *Proteobacteria* in sugar-cane cultivated soil, and a diverse pool of genes potentially involved in metabolic process and environmental interactions.

Key words: Diversity, DNA Sequencing, Sugar Cane, Microrganism

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P143
Diversity and structure of the soil microbiome under long-term organic and conventional farming

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Sustainable farming practices are essential to maintain ecosystem functionality, preserve biodiversity, and meet economic demands. In this light, the Swiss DOK long-term agricultural management experiment has been initiated in 1978 in order to compare biodynamic, bio-organic, and conventional farming practices. Initial research focused on feasibility and productivity of organic farming, but more recent emphasis has been given to farming-related effects on soil quality. Since microbial processes regulate soil ecology and biogeochemistry, microbial diversity and community structure might serve as indicators of sustainable management.

We have previously reported on findings based on genetic profiling (T-RFLP and RISA) of microbial ribosomal loci. These data have revealed consistent effects of long-term farmyard manure application and short-term crop cultivation; however, effects were more consistent for bacteria than for fungi. A classical sequencing approach yielded indications for specific bacterial groups that responded to the management influences, but insufficient diversity coverage and sample throughput did not allow for determining robust management indicators. Recent developments in sequencing technologies stimulated a reassessment of this site at much higher resolution and we re-analyzed the same soil samples using a massively parallel pyrosequencing approach targeting bacterial (16S) and fungal (ITS) ribosomal markers.

We recovered a total of 1,118,268 bacterial and fungal pyrotags from 80 soil samples, revealing predominant abundances of Actinobacteria (30%), Proteobacteria (29%), and Acidobacteria (10%) as well as Ascomycota (53%), Basidiomycota (17%), and members of the former Zygomycota (17%). In general, massive sequencing supported the results from the previous studies, but revealed a substantial increase in resolution by identifying effects that went undetected using the traditional profiling approaches. Fertilizer application, crop protection regime, and crop cultivation were identified as major factors driving the structure of the soil microbiome. Network association analysis identified a range of microbial taxa that were significantly affected by the management practices, providing a first basis to elucidate long-term farming effects on soil quality.

P144
Metagenomic analysis of arctic marine microbial communities

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The Arctic Ocean is currently undergoing environmental changes due to global warming and this is especially evident looking and the increase in temperatures and decrease in summer sea ice. The marine microbial community plays a key role in the global ocean carbon cycle but still little is known about the specific functions they conduct. The purpose of this study is to investigate the functions of the arctic marine microbial community. Samples were obtained from the Arctic Ocean at three different depths (surface, medium and deep) together with marine samples from the South Pole. Total DNA was purified and sequenced using the Illumina sequencing technology. After assembly of the arctic marine metagenomes over 5 million genes were identified and compared across samples. This comparative gene analysis reveals functional niches of the different environments investigated in this study and high gene diversity.

P145
Effects of rainfall events on microbial processes in forest soil

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Decomposition of dead plant biomass is a complex process including mineralization of organic compounds and formation of soil organic matter and thus affecting the carbon balance in terrestrial ecosystems. Performed by extracellular enzymes, these processes require interaction among a variety of microorganisms whose presence and activity are subject to regulation by various environmental factors. The major physical factors are soil moisture and temperature which drive C turnover in soil and act indirectly - mainly by affecting microbial activity that drives the mineralization of soil organic matter and plant residues. The aim of this study was to describe dynamic changes in microbial activity in upper layers of forest soil after simulated rainfall events.

Soil cores (25 mmdiameter,100 mmdepth) were collected before the onset of the vegetation season. The cores were exposed to a

simulated rainfall of intensity8 mmper hour (3 ml per core) and subsequently incubated at12°Cfor 15 days. Respiration was monitored continuously. Cores were collected at 0, 1, 3, 5, 7, 10 and 15 days. Soil core material was separated into 5 mm thick slices up to a depth of4 cm. Titre of extracellular enzymes (cellobiohydrolase, β-glucosidase, β-galactosidase, N-acetylglucosaminidase, β-xylosidase, α-L-arabinosidase) were assayed by direct incubation of soil with 4-Methylumbelliferyl-based substrates. Microbial biomass was quantified by ergosterol measurement and qPCR.

The soil moisture increased by 40-10% in the upper layers of soil cores after water addition. No significant changes in respiration were detected in moistened cores. Change of soil moisture, however, affected the production of extracellular enzymes and biomass. Strongest effects were observed in the upper parts of the core and the influence decreased with soil depth. The results show that rainfall events have a strong effect on enzymatic decomposition of organic matter, especially in the uppermost parts of the forest soil.

P146

Rhizosphere and bulk soil microbial communities affected by soil type but not by biocontrol strain *Pseudomonas jessenii* RU47

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The soil borne pathogen *Rhizoctonia solani* is hard to control with fungicides and the use of antagonists could be a suitable control strategy. *Pseudomonas jessenii* RU47 showed reproducible disease suppression effects against *R. solani* on lettuce in pot and field experiments. In this study we aimed to investigate the effect of the soil type on the rhizosphere competence and biocontrol efficiency of RU47 on lettuce and on microbial community composition in a unique plot system containing three soil types (Diluvial sand, Alluvial loam and Loess loam). The experimental approach made it possible to analyze the complex interactions between the soil type, plant and microbial community independently from factors such as climate and cropping history. The experiment was performed with lettuce in the presence of the pathogen *R. solani* and the biocontrol strain *P. jessenii* RU47 over two seasons. The dry weight and disease severity of lettuce was analyzed as well as the rhizosphere colonization of the biocontrol strain RU47. Total community DNA from rhizosphere pellets and bulk soil was analyzed by molecular fingerprints (DGGE, amplicon pyrosequencing of 16S rRNA genes or ITS). Significant differences in the bacterial or fungal community composition depending on the soil type were observed while no effects of the inoculant or the presence of the pathogen were found. PCR-DGGE analysis of the 16S rRNA genes of *Alpha-* and *Betaproteobacteria* and *Actinobacteria* also showed that the rhizosphere microbial communities of lettuce in the three soil types differed significantly whereas the biocontrol strain had only a negligible effect on the indigenous microbial community in both seasons. Pyrosequencing revealed that the dominant phyla in bulk soil and lettuce rhizosphere of all three soil types were *Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria* and *Bacteroidetes.* Analysis of the OTU report showed that several taxa belonging to different genera of the *Proteobacteria* (*Acidovorax*, *Sphingomonas* and *Rhizobium)* were enriched in the rhizosphere of lettuce independent from the soil type. Our data indicate a strong selection of *Proteobacteria* by lettuce grown in different soil types providing an explanation for the high rhizocompetence of RU47 in all soil types.

P147

Application of biocontrol strain *Pseudomonas jessenii* RU47 did not impact the fungal community in three different bulk soils

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The soil borne pathogen *Rhizoctonia solani* is hard to control. The use of fungicides is critical with respect to pesticide residues in the final product. Hence, the use of antagonists could be an environmental friendly control method. A promising biocontrol strain was isolated from a disease suppressive soil and was already tested under growth chamber and field conditions. This strain was identified as *Pseudomonas jessenii* RU47. To evaluate its biocontrol efficiency, a field experiment with a unique plot system containing three soil types was performed. For this lettuce plants were grown in three different soil types with or without the presence of the fungal pathogen *R.solani* and inoculated with the RU47. Previous studies showed that RU47 is able to produce proteases and hydrogen cyanide, both known for antifungal activity. As the antifungal effect of RU47 also against *Fusarium oxysporum* is known, we expected an impact of the RU47 on the fungal community in the bulk soil after two successive lettuce growth periods with repeated RU47 applications. To study the influence of the biocontrol agent on the fungal community DGGE and pyrosequencing analyses of ITS fragments amplified from total community (TC) DNA were carried out. Total community DNA was extracted from the bulk soil of all three soil types, 4 replicates each. The following DGGE analysis showed that the bulk soil microbial communities in the three soil types differed significantly whereas the biocontrol strain RU47 and the presence of *R. solani* had no effect on the indigenous fungal community. For a more detailed analysis the ITS fragments amplified from TC-DNA of the bulk soil were also analyzed by pyrosequencing. The dominant genera of fungi were *Fusarium* in all three soil types, followed by *Cryptococcus* and *Plectosphaerella*. In accordance to the DGGE results soil type dependent differences in taxonomic composition were observed. The variability of the sandy soil was higher than in the two loamy soils. A nonmetric multidimensional scaling based on the Bray Curtis dissimilarity showed no difference between the control plots and the plots with the RU47 application. Our data indicating that the biocontrol strain RU47 had no impact on the fungal community in the bulk soil after two successive lettuce growth periods with repeated RU47 applications.

P148

Prokaryotic diversity of wetland evaluated both by isolation and cultivation-independent analyses

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Culture-dependent Background and -independent analyses were carried out to investigate the diversity of bacterial community in water layer of Woopo wetland, Changnyeong, Gyeongnam, Korea. Amplified rDNA restriction analysis (ARDRA) was applied onto both of the isolates and 16S rRNA gene clones obtained directly from DNA extracts. Representative isolates and clones of all the single isolate/clone phylotype were partially sequenced and analyzed phylogenetically. Denaturing Gradient Gel Electrophoresis (DGGE) accompanied by sequencing and pyrosequencing were applied to estimate the seasonal variation of bacterial community. Sixty-four and 125 phylotypes were obtained from 203 bacterial isolates and 235 culture-independent 16S rRNA gene clones, respectively. Bacterial isolates were composed of 4 phyla, of which *Firmicutes* (49.8%) and *Actinobacteria* (32.0%) were predominant. Isolates were affiliated with 58 species and among them 30 strains were presumed to be a novel species. Culture-independent 16S rRNA gene clones were composed of 8 phyla, of which *Proteobacteria* (62.2%), *Actinobacteria* (15.5%) and *Bacteroidetes* (13.7%) were predominant. DGGE profile in spring was distinguished from that of rest seasons. But, in all season, *Proteobacteria* was the most predominant phylum followed by *Actinobacteria*, *Bacteroidetes* and *Firmicutes*. Total bacterial reads, 16,350 with average length of 450 bp were obtained by pyrosequencing of partial 16S rRNA genes. The higher diversity was found in Spring, which was also expressed by the largest number of phyla present and only one genus exceeding 5 % of total members. *Proteobacteria* was the most predominant phylum (Summer, 60.8 %; Autumn, 49.4; Winter, 41.6; Spring, 36.9) followed by *Bacteroidetes* (16.1 - 29.3 %) and *Actinobacteria*, (6.8 - 25.9 %) but its proportion varies seasonally. A lager amount of members of phylum *Actinobacteria* was found in Winter and Spring. In particular, the genus "*Planktophila*", a candidate member of *Actinobacteria* was the most abundant genus in Spring. High concentration of chlorophyll a and presence of large number of bactreioplankton "*Planktophila*" in Spring suggests that isolation and characterization of the microorganism is urgent and taking an another viewpoint of water bloom is necessary.

P149

Influence of pharmaceuticals on composition of 16S rRNA and bacterial laccases genes in wastewater treatment bioreactors

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Introduction: Laccases are multicopper oxidases that oxidise a wide variety of phenolic compounds and are thus environmentally and industrially important enzymes that may be involved in degradation of pharmaceuticals. Fungal laccases were shown to potentiate removal of pharmaceuticals and we found that pharmaceuticals influence bacterial community composition (Kraigher *et al.*, 2009). However, there is no information on how removal of pharmaceuticals, composition of 16S rRNA and bacterial laccase genes are linked, which was the aim of this study.

Methods: To address these questions, two types of bioreactors exposed to wastewater rich in pharmaceuticals were studied: 1) bioreactors with moving bed biofilms and 2) suspended sludge bioreactors. Bioreactors treated only with artificial wastewater were used as controls. For both systems exposed to pharmaceuticals data on their removal was collected (Kosjek *et al.*, 2012; Zupanc *et al.*, 2013) and community composition was studied at the level of 16S rRNA and proteobacterial laccase genes with DGGE and gene sequencing.

Results: Our results show that pharmaceuticals decreased bacterial diversity at the level of 16S rRNA. Bacterial community was also more stable in control reactors as compared to the treated reactors over time. No major differences in bacterial communities were observed between bioreactors with different performance. Proteobacteria were dominant in all examined reactors with *Betaproteobacteria* and *Chloroflexi* being most abundant in suspended sludge reactors and *Gammaproteobacteria* in moving bed biofilm reactors. DGGE profiles of proteobacterial laccase genes showed high diversity in all reactors. Composition of bacterial laccase genes varied over time and was dependent on the type of bioreactor but application of pharmaceuticals did not shift the composition.

Conclusions: We have observed a distinct shift in bacterial community over time in bioreactors supplied with pharmaceuticals in comparison to control; however, according to the DGGE analysis proteobacterial laccases seem not to be subjected to the effect of pharmaceuticals.

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P150

Changes in microbial communities in alpine soil exposed to increased nitrogen and ozone levels

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Adverse ozone and nitrogen effects on vegetation have been reported, resulting in a reduction of agricultural productivity as well as in changes of biodiversity. However, little is known about effects of these air pollutants on soil microbial organisms. Bacteria and fungi fulfill important ecological functions in soil and therefore contribute to the quality of soil. The goal of this study

was to analyze the effects of increased levels of ozone and nitrogen on soil microbial community structures.

Soil samples were collected from monoliths located in a subalpine grassland ecosystem at 2000 m above sea level at three different seasonal time points between May and August in 2010. The monoliths were treated with 1.6 × ambient ozone concentration (O), 50 kg N $ha^{-1}y^{-1}$ (N), as well as the combination N×O or left untreated, starting in 2004. Soil microbial community structures were characterized using ribosomal intergenic spacer analysis (RISA) and massively parallel pyrosequencing of the 16S (bacteria) and ITS (fungi) rRNA region.

Analyses based on length polymorphism (RISA) and sequence composition (pyrosequencing) were significantly correlated for both bacteria and fungi. RISA recovered a total of 44 bacterial and 31 fungal operational taxonomic units (OTUs), respectively. Both bacterial and fungal community structures were significantly influenced by the treatments, explaining 5.2 and 6.5% of the variance. For fungi, O and N×O were major drivers of the community structure, whereas the bacterial community was much stronger influenced by the temporal component.

High-throughput pyrosequencing generated 207,666 bacterial and 273,311 fungal sequences, resulting in 4,632 bacterial and 1,555 fungal OTUs at 97% sequence identity. In accordance with the RISA results, the treatments significantly altered both communities, explaining 14.7 - 21.3% of the variance. Fungal community structures were mainly influenced by N, O, and N×O, while alterations in the bacterial community were driven by N×O only. Several individual bacterial and fungal taxa were found to be significantly associated with particular treatments. These findings indicate the presence of potential indicators for increased levels of nitrogen and ozone levels and may help to identify possible changes in soil functions due to these air pollutants.

P151
Resistance and resilience of the forest soil microbiome to soil compaction after logging operations

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Soil compaction has been recognized as a major disturbance associated with logging operations, but we lack fundamental knowledge how this affects the soil microbiome. We assessed resistance and resilience of the soil microbiome after compaction and correlated our findings with changes in soil functions. Logging traffic across a soil moisture gradient installed at two different forest sites generated replicated skid trails of different impacts. Soil physical properties and fluxes of greenhouse gases were measured to assess alterations in soil functioning in these skid trails. Metagenomic DNA was extracted from soil samples collected at various time points after compaction in order to assess microbial diversity and community structure using massively parallel pyrosequencing of bacterial and fungal ribosomal markers.

The analysis of about 900,000 pyrotags revealed that compaction significantly altered diversity and structure of both bacteria and fungi. The strongest effects were observed in severely compacted soils where air and water conductivities dropped below 10% of the initial value. Sandy soils revealed higher resistance to compaction than clayey soils. Effects were most pronounced in the medium-term (180-365 days) and were less strong in the short- and long-term (30 days or 4 years), but communities in the severely compacted soils did not yet show resilience after 4 years. Taxa-treatment association analysis revealed that anaerobically respiring bacteria (e.g. sulfate- and metal-reducers) from the Firmicutes, Delta- and Betaproteobacteria as well as fungal saprobes from the Ascomycota were increased in compacted soils. Conversely, aerobically respiring bacteria from the Actinobacteria, Alpha- and Gammaproteobacteria as well as mycorrhizal fungi from the Basidiomycota were negatively affected by compaction. Accordingly, greenhouse gas fluxes significantly changed in the compacted soils, resulting in reduced carbon dioxide and increased methane and nitrous oxide emissions.

This study demonstrates that physical soil disturbance during logging alters soil functioning and that the response of the microbiome is massive and tightly linked to these changes. Taxa indicative of these conditions can now help to monitor resistance and resilience of various soil types after logging operations.

P152
Exploiting and bioprospecting Brazilian biomes for phosphorus disponibilization

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Brazil is known by its native biodiversity, mainly explored in terms of plants and animals. However, another piece of Brazilian biodiversity, still scarcely explored and used, is the microbial diversity present in the distinct biomes found in this country. The though description of such biodiversity is highly desirable, and the bioprospection in such environments may elect new names to perform a vast variety of processes, serving as a black box of bioresources for biotechnology. Here we elected two biomes (one endemic in Brazil) to explore the presence of microbes involved in the availability of phosphorus, on its mineral forms, in these environments. Mangroves are particular environments, located in the interface between land and sea, where the frequent conditions of anaerobiosis lead to the accumulation of organic matter in such soils. It combined with the salinity might select unique organisms involved in the organic matter degradation, possibly releasing mineral phosphorus from organic sources. In counterpart, the caatinga is characterized by the location in the semiarid region in Brazil, where the low availability of water, together with the occurrence of high temperatures lead to the low contents of organic matter in such soils. In these areas, the major role of microbes might be in the release of phosphorus from insoluble sources (combined with calcium and aluminum). Our approaches revealed the presence of organisms related to these functions in both biomes (cultivation-based analyses), and also indicated the occurrence of genes related to these processes (*bpp* and *pqqE*) in the microbial community residing in such soils (culture-independent analyses). Although initial, these results confirm the possibility to explore these sources for microbes

performing presenting features. Further analyses will name and evaluate the endemicity of such organisms and genes in the exploited areas.

P153
List and ecological information of bacteria with valid names, isolated from Republic of Korea

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More than eight hundred and fifty novel bacterial strains were isolated and validly published with names until Dec. 2012. Construction of database including taxonomic rank, physicochemical properties and ecological information of the isolates was needed to manage them as biological resources. The published papers on the novel strains isolated fromRepublicofKoreawere screened using the journals dealing microbial taxonomic affairs. The scientific names of the isolates were confirmed using the approved list described in internet homepage (http://www.bacterio.cict.fr).Eight bacterial phyla were reported on the isolates: 365 Proteobacteria, 214 Bacteroidetes, 168 Actinobatera, 92 Firmicutes, 6 Deinococcus-Thermus, 2 Verrucomicrobia and 1 Lentisphaerae. In addition to bacteria, 6 Archaea species were reported.Isolation of the novel species was achieved from various sources: air (25), compost (26), endophyte (4), rhizosphere (22), fermented seafood or Kimchi (11), freshwater lake, river or wetland (53), ground water (4), soil (228 including ginseng soil (59)), Tidal flat sediment (177), seawater (144), marine animal (24), seaweed (11), wastewater or sewage (41), solar saltern (49), e.t.c (31). Marine bacterial species accounted half of the total novel species. The highest taxonomic rank of the novel strain originated from Koreais the class: *Fimbriimonadia* class nov. (phylum *Armatimonadetes*).

P154
Efficacy of ozonized water for microbial decontamination of fruits

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Fruit contamination with bacteria and microfungi is harmful as it often reduces fruit quality and presents a potentially serious health risk to humans when fruits are used in candy factory. The percentage of external contamination by bacteria and microfungi was evaluated. Thirteen fruits were tested for fungal and bacterial contamination. The contamination of the fruits with microfungi ranged from 4.71 $X10^2$ -3.5$X10^4$ CFU/g and are arranged in descending order as follow: peach>strawberry>apple>nectarine>pears>cherrie>grapes>raspberries>pinapples>mangoes>bananas>kiwi>papaya. The most common fungi recovered from the fruits were *Penicillium expansium*, *Aspergillus niger*, *Rhizopus oryzae*, *A. flavus*, *A.fumigatus*, *Acremonium strictum*, *Ulocladium chartarum*, and *Alternaria alternate*. Bacterial contamination on fruits ranged from 3.1 $X10^3$ -1.7$X10^5$ CFU/g. *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Bacillus cereus*, and *Staphylococcus aureus* were common on peach, nectarine, pinapple, kiwi, and papaya, respectively. One ppm of ozonized water was sufficient for 100% microfungal decontamination while the bacterial count was reduced by 83-91%. Complete decontamination of bacteria was achieved on washing with 2 ppm ozonized water. These results suggest that the ozonized water can be useful as a hurdle for extending the shelf life of fruits during storage.

P155
Microbiology of cave sediments - is oligotrophy all that matters?

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Cave microbiology, recently acknowledged as an integral part of speleology, has yet to determine the structure of microbial communities and its relation to the subsurface environment. Due to 43 % of surface as karst and 10419 caves registered to this day, Slovenia has ideal settings as well as responsibility to invest in research of these delicate environments. The purpose of this study was to asses the difference in microbiological and environmental properties of clastic sediments between four sampled caves in comparison to the surface soil. In addition, incubation experiments were used to model the spring-autumn inflow of nutrients and organisms into cave sediments. Finally, a sediment transect spanning significant differences in cave conditions encompassing also sediment age up to sediments from Middle Quaternary (<0.78 Ma) was analyzed. Within-cave sediment characteristics variability was higher than between cave variability, suggesting high spatial heterogeneity of environmental conditions within each particular cave. The variability in bacterial microbial communities followed that of the sediments, but was significantly different from surface soil community. Incubation experiments showed that despite the influx of cell and nutrients into cave sediments, the microbial community structure remained characteristics of cave environment. In addition, characteristic communities resembling cave microbial community structure developed on sterilized glass beads incubated under the same conditions. These findings were corroborated by the results of Middle Quaternary transect. In order to determine the robustness of these observations, global comparison of 16S rRNA sequences of microbial communities from deep subsurface, mine, cave sediments, polluted soils, cold, polar, alpine and extreme soil, rock endoliths and temperate soils was conducted. With increasing breadth of environments from extremely oligo- to nearly eu-trophic with primary production it became evident that cave bacterial communties did not represent a specific subterranean cluster but were largely similar to other sediment-soil communities with limited substrate supply and low temperatures.

P156

Bacterial diversity and soil physiology dynamics in no-till agriculture soils under different managements

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No-till is a conservation farming practice adopted in several parts of the world to cope with soil erosion, increase carbon storage and make a more efficient use of available moisture. However, farmers' experience suggests that reduced tillage needs to be coupled with crop rotation and good agrochemical management to be sustainable. Samples were obtained in Summer and Winter during 2010 and 2011 in no-till soils managed with two contrasting practices (high or low crop rotation), and a grassland as control (3 treatments), replicated in four geographical sites (4 different soil textures) located across a regional scale of 400 km in the Argentinean Pampas (3 subsamples for each case). For the comparison of the bacterial community structure a fragment of the v4 region of 16S rRNA gene was high-throughput sequenced. Soil physiology was studied by: FAME analysis of Phospho and Neutral fractions of soil lipids; characterization of Physiological Profile at the Community Level (CLPP) using substrate induced respiration, characterization of Phosphatase activity, and estimation of Glomalin related soil proteins. > 150,000 16S rRNA gene sequences were obtained for a total of 108 samples. We did not find significant differences in bacterial diversity that can be related to sites or soil management. However, there are phylogenetic differences between samples, which can be explained on the basis of both geographical location and agricultural management. Fame analysis shows that PLFA separates samples geographically while NLFA does it by soil treatments. Branched chain FA/PUFA and MUFA/PUFA ratios of Neutral Lipids discriminate agricultural practices. FAs 20:0 and 18:1w7c appear to be markers for different soil managements independently of the season. CLPP analyses show a sharp discrimination between different soil treatments being clearer in winter samples. Phosphatase activity analyses show equal results although discrimination between treatments was better in summer samples. Glomaline related soil proteins show different variations according to soil management depending on the soil texture or environment. The integration of all these data will give the basis to learn how to maintain the sustainability of agricultural soil ecosystems and to find biomarkers for diagnosis of soil quality within the Pampa region.

P157

Microbial diversity and transformation of organic pollutants on distinct soil particle surfaces

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Soil microorganisms live in close contact with surfaces provided by particulate organic material (POM) and organo-mineral complexes, the latter composed of different particle size fractions (PSF), i.e., sand (diameter 63 - 2000 µm), coarse silt (20 - 63 µm), fine silt (2 - 20 µm) and clay (< 2 µm). Soil organic carbon (SOC) differs in quality and quantity depending on the PSF. In previous studies we could demonstrate by T-RFLP of PCR-amplified SSU rRNA genes that soil fractionation based on mild-ultrasonication and centrifugation, allowed to isolate sand-sized, silt and clay fractions with structurally distinct communities of bacteria, archaea and fungi (Neumann et al., 2013). Using the example of SOC variants from two long-term fertilization field sites (Bad Lauchstädt, Germany; Askov, Denmark) it was found that the responsiveness of the microbial communities to different fertilization regimes was high in the larger sized-fractions but declined with smaller down to a non-detectable effect at clay. Considering that the heterogeneous surface properties of soil constituents also affect the partitioning and sorption of organic pollutants, we hypothesize that after entering a soil, different amounts of pollutant will interact with distinct microbial communities on PSF. Using the example of phenolic compounds we found that the sorption of phenol declined with increasing levels of SOC at the clay fraction, while SOC of the other fractions had no effect. In contrast, the sorption of 2,4-Dichlorophenol (DCP) increased with SOC, mainly due to sorption by POM. Studies with ^{13}C-labeled compounds and stable isotope probing (SIP) on the non-fractionated soil variants showed that depending on the fertilization regime, different bacterial taxa were involved in the degradation of both phenolic compounds. We could now demonstrate that after the fractionation procedure, the microbial cells of the PSF were still metabolically active and that the catabolism of the microbial communities from each PSF can separately be studied. Results from these studies should provide novel insights in the existence of catabolically distinct communities on soil particle surfaces.

Reference

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P158

The probiotic potentials of some lactic acid bacteria from some african fermented foods

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The probiotic potentials of lactic acid bacteria species isolated from various food sources (nono, ugba, ogiri, kunun-zaki and ogi) were studied. Biochemical tests and mol G+C content were employed to ascertain the identity of the isolated strains. The predominant species among the isolated strains were *Lactobacillus bulgaricus, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus brevis, Streptococcus thermophilus Leuconostoc mesenteroides* and *Pediococcus cerevisiae*. The probiotic properties assessed were bile resistance, acid tolerance and ethanol tolerance. In vitro results obtained showed that three strains *Lactobacillus bulgaricus, Lactobacillus plantarum* and *Streptococcus thermophilus* were able to meet the basic requirements for probiotic functions such as tolerance to pH 3, growth in 0.5%(v/v) bile salt and ethanol at 5%(v/v). The isolates had low hydrophobicity property, ability to degrade raffinose and stachyose and coagulation of milk. The isolates also showed amylase activity. They produced bacteriocins that inhibited the growth of *S. aureus*, *E. coli* and *B. cereus*. The mean lactic acid and hydrogen peroxide were 4.78±0.31 and 1.72±0.34g/100ml respectively. KEY WORDS: PROBIOTICS, LACTIC ACID BACTERIA, BILE RESISTANCE, ACID TOLERANCE, ACID TOLERANCE.

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P159
The indoor microbiome: diversity and its control by beneficials?

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Although indoor environments provide residence to numerous specific microbial communities, little is known about the microbial diversity inside of these communities and its impact on human health. We studied indoor microbioms in intensive care units in hospitals as well as alternative possibilities to manage these microbial communities.

In the intensive care units (ICUs), we used amplicon pyrosequencing to study the ICU microbiome and were able to detect diverse sequences, in comparison to the currently used standard cultivation technique that only detected 2.5% of the total bacterial diversity [1]. The phylogenetic spectrum combined species associated with the outside environment, taxa closely related to potential human pathogens, and beneficials as well as included 7 phyla and 76 genera. In addition, *Propionibacterium* spp., *Pseudomonas* spp., and *Burkholderia* spp. were identified as important sources of infections. Despite significantly different bacterial area profiles for floors, medical devices, and workplaces, similarities by network analyses and strains with identical molecular fingerprints were detected. Moreover, on the basis of these results, beneficals with antagonistic activity, new bio-based antimicrobials, the use of light-activated disinfection for specific surfaces and clothes were developed, which allow managing "healthy" indoor microbiomes [2,3].

This information will allow for new assessment of the interaction with our surrounding microbiome but also of sterility in more general.

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P160
Evaluation of bacterial nanocellulose membranes – activity and stability study of the antimicrobial nisin peptide

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Introduction: The incorporation of antimicrobial, such as nisin, in bacterial cellulose has a wide applicability in pharmaceutical, medical, chemical, cosmetic, food and other areas. Nisin is a natural antimicrobial peptide used as food preservative; being effective at controlling a broad range of Gram-positive bacteria, including the multidrug-resistant pathogen. Bacterial cellulose (BC) production using waste as culture media is a novelty, stimulating scale-up for industrial production and extended applications.

Objectives: the objective of this work was to evaluate the nisin activity after incorporation into BC standard and BC produced by using food waste.

Materials and Methods: BCs were submersed in 1mL of 250 µg nisin (Sigma®, 1g contain 2.5% of nisin), in phosphate buffer saline (PBS) pH 4.5 (sterilized by filtration, 0.22 µm). After absorption, at pre stablished parameters, BCs were kept under refrigeration at 4ºC and nisin activity was determined, by agar diffusion assay with *L. sakei* as bioindicator, at different periods, from 1 to 45 days.

Results: Our results indicated that all BCs were capable to maintain the nisin activity up to 45 days of storage, and kept high activity.

Conclusion: Our studies highlighted the importance of an effective antimicrobial system able to assure safety and stability to pharmaceutical and medical products. On a near future, the BCs combined with nisin will be tested against Gram-positive and Gram-negative microorganisms, and its possible cytotoxicity effects.

Keywords: bacterial cellulose, *G. xylinus*, nisin, antimicrobial, nanocellulose, biomaterial.

P161
Exploitation of PGPB and soil amendments in the phytoremediation of heavy metal contaminated sites

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Soil contamination with cadmium, lead, and zinc is one of the most pervasive environmental problems. In the surroundings of a former Pb/Zn smelter in Arnoldstein (Austria) heavy metal concentrations in planted crops exceed thresholds for usage as food and feed. Phytoremediation (in our case the combination of immobilization and phytoexclusion) could be a cost-effective system for the improvement of the use of this contaminated area. The aim was to study the effects of plant growth-promoting bacteria (PGPB) and immobilizing soil amendments on heavy metal tolerance of plant and uptake. Pot experiments were performed whereby two maize cultivars were cultivated in different contaminated soil and treatments (*Burkholderia phytofirmans* strain PsJN with and without amendment). PsJN transformed with the beta-glucuronidase (GUS) reporter gene was used to allow a PCR based detection of the strain in miaze shoots. Inoculation with strain PsJN significantly improved root and shoot biomass of maize. Rhizosphere and leaves were analyzed for heavy metal content. Results indicated that immobilizing amendments had significant effects on reduction of ammonium nitrate extractable Zn (< 80%) and Pb (<50%) compared to the controls. Concentration of Zn and Pb in plants was reduced by combined immobilizer and PGPB up to 65% and 40%, respectively.

Simultaneously, three different media allowed the selection of 350 bacterial isolates comprising rhizosphere bacteria and endophytes based on colony morphology. 16S rDNA sequence based identification of bacterial isolates revealed the presence of bacterial groups such as Actinobacteria, Firmicutes, Gamma-Proteobacteria, Bacteroidetes and Beta-Proteobacteria. Amplification of 16S-23S rRNA intergenic spacer (IGS) region followed by fragment analysis by gel electrophoresis was performed. Furthermore, established the presence of different strains within *Pedobacter ginsengisoli, Rhodonobacter fulvus, Microbacterium lacticum, Arthrobacter sp* and *Methylobacter sp.* The plant growth-promoting potential was analysed by screening for the production of 1-aminocyclopropane-1-carboxylic acid deaminase, heavy metal tolerance and metal mobilization. Selected strains will be tested for plant growth-promoting effects in interaction with the plant also in field.

Keywords: Immobilization, Phytoremediation, Treatment, Heavy metals, 16S rDNA, IGS typing

P162
Gut microbiota, host energy metabolism, and potential probiotic effects

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The human gut microbiota has been implicated a crucial role in regulating host energy metabolism; and changes in its community composition have been documented alongside with metabolic syndrome related disorders. Thus, novel strategies for prevention and therapy of metabolic diseases may involve the targeted modulation of the gut microbiota community. Our study examined how the bacterial communities in the gut relate to parameters of the lipid and glucose metabolism in patients with impaired metabolic functions. Furthermore, it explored potential effects of daily consumption of a probiotic drink containing *Lactobacillus casei* Shirota on energy metabolism and the composition of the gut microbiota. In a cross-over-study with daily intake of one portion of a probiotic drink containing *L. casei* Shirota, fasting blood samples were collected, anthropometric parameters were measured and a frequently sampled oral glucose tolerance test was performed. Effects of the probiotic drink on the human gut bacterial communities were studied via 16S rRNA based T-RFLP fingerprinting and quantitative PCR analysis of specific bacterial groups. Clinical analyses did not evidence any beneficial probiotic effects on lipid and glucose metabolism except a small reduction in fasting glucose levels, and even imply a negative impact on cholesterol and triglyceride metabolism. No consistent changes in the gut microbiota studied via 16S rRNA gene community profiling were observed following 12 weeks daily consumption of the probiotic drink. However, in some cases, shifts in specific phylotypes were detected, which are presently analyzed via sequencing and qPCR analysis of individual bacterial groups.

P163
Comparative genomics and antimicrobial activity of *Lysobacter* species

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Lysobacter species are Gram-negative bacteria that are widely distributed in diverse ecosystems, including soil, rhizosphere and freshwater habitats. Various members of this bacterial genus have demonstrated antagonistic activity against a range of other (micro)organisms, including bacteria, fungi, oomycetes and nematodes. They produce a variety of extracellular enzymes and antibiotic-like compounds, most of which have not been structurally identified. Several *Lysobacter* strains have been isolated from soils suppressive to *Rhizoctonia solani,* a devastating fungal pathogen of numerous economically important crops such as sugar beet, potato and rice. These *Lysobacter* isolates showed expressed chitinase and β-1,3-glucanase activity, and strong *in vitro* activity against *R. solani* and also other pathogenic fungi, oomycetes and bacteria. To date, the bioactive compounds involved in the antimicrobial activity of *Lysobacter* species and corresponding genes remain elusive. In this study, draft sequences were determined for the genomes of three *Lysobacter* species, including *L. antibioticus*, *L. capsici* and *L. gummosus*. Comparative genomics is currently being performed to find differences between the species, and to explore these genomes for genes or gene clusters involved in the biosynthesis of antimicrobial compounds. In parallel, transposon mutagenesis is performed to identify the genes involved in *in vitro* activity against *R. solani*.

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Bacteria isolated from the deep-sea hydrothermal field of Kolumbo submarine volcano – a potential source of new bioactive compounds

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The escalating problem of drug resistance, especially in the treatment of infectious diseases, possesses urgently the need for new drugs. Over the past 25 years, more than 50% of all new chemical entities approved as drugs were based on natural products. Microbes in hydrothermal vents with their unique secondary metabolism represent an untapped potential source of new types of natural products. In this study, sediments were collected from the hydrothermally active field of Kolumbo submarine volcano (500 m depth), in the Aegean Sea, during E/V Nautilus 2010 expedition, in order to isolate mesophilic bacteria with antimicrobial activity. Samples were plated on selective media and incubated aerobically for 7 days at 25 °C. The isolated bacteria were then differentiated through BOX-PCR analysis at the strain level and antimicrobial activity was tested by diffusion method. Bacterial isolates grown on agar plates were coated with 0,7% soft agar containing 18 different type strains (pathogenic and non pathogenic) of Gram positive and negative bacteria, fungi and yeasts. 832 aerobic heterotrophic bacteria were isolated and corresponded to 230 different BOX-PCR genomic fingerprints, 189 of which showed remarkably reproducible antimicrobial activity. The most bioactive isolates, 45 in total, that showed antimicrobial activity against at least 6 type strains, were then selected for sequencing and phylogenetic analysis. The future work will be focused in the isolation and identification of the bioactive compounds produced by the selected isolates.

P165

Microalgae from Greek environments: Potential use for biodiesel production

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With energy prices increasing constantly, biodiesel as an alternative fuel is attracting more and more attention. Currently, the most common feedstock for biodiesel is pure vegetable oils. Their availability though is reduced and new sources are being investigated. Microalgae have been recognized as potentially good sources for biodiesel because of their high lipid yield and poor growth factor demands. The objectives of this study were to isolate microalgae from interesting Greek habitats that would have the ability to produce lipids suitable for biodiesel. Ecosystems that suffer from atmospheric pollution such as the monuments of Acropolis and the coast of Aspropyrgos, were selected. The isolated microalgae were maintained in Walne medium. They were screened for biomass production and those with maximum efficiency were selected for lipid extraction followed by Gas Chromatography analysis. Two strains with both high biomass and lipid yield were selected (*Chlorella* sp., and *Blindingiua minima*) and their growth conditions (pH, temperature, nutrients) were optimized in 20 L cultures. Under nitrogen starvation both strains showed higher lipid production. Using the optimum conditions, semi-pilot scale cultures (30 L) were made and high biomass (5 g/L and 3 g/L) and lipid production (700 mg lipids/L and 600 mg lipids/L) for the two strains respectively was achieved. In conclusion, the lipid production was sufficient and further modifications of the growth factors could lead to improved yields.

P166

Oil-degrading rhizosphere and endophytic actinomycetes to enhance the phytoremediation ability of corn plants in oil-polluted soil inthe United Arab Emirates

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The ability of oil-degrading rhizosphere and endophytic actinomycetes to promote the growth of corn plants in soil polluted with crude oil in the United Arab Emirates was evaluated under gnotobiotic and greenhouse conditions. These 23 isolates of rhizosphere actinomycetes and 15 isolates of endophytic actinomycetes were selected based on their ability to produce plant growth regulators (PGRs) incldуing auxins, cytokinins, gibberellins and polyamines and through the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Under gnotobiotic and greenhouse conditions, the application of a mixture of five actinomycetes which exhibited the maximum production of PGRs and ACC deaminase promoted corn roots and shoots in oil- polluted soils comapred to control plants grown in oil-polluted soils without the application of the actinomycetes. The application of these five actinomycetes isolates has also significantly increased photosynthetic pigment contents, photosynthetic carbon assimilation, plant water use efficiency and promoted corn growth characteristics including increased fresh and dry weight and length of roots and shoots, total leaf area compared with control seedlings. The application of a mixture of actinomycetes also significantly, reduced the levels of ACC in the roots and shoots, and significantly increased the levels of indole-3-acetic acid, putrescine, spermidine and spermine in roots and shoots compared with control plants grown in oil-polluted soils without the application of actinomycetes. The application of a mixture of actinomycetes significantly, reduced the levels of the total recoverable hydrocarbons and polycyclic aromatic hydrocarbons in oil-polluted soils compared with control treatment. This study is the first report to demonstrate the potential of oil-degrading rhizosphere and endophytic actinomycetes to improve the phytoremediation capability of plants grown in oil contaminated site through the production of ACC deaminase and PGRs.

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Genome-based optimization of the biocontrol performance of the sugar beet endophyte *Pseudomonas poae* RE*1-1-14

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Genome sequence information of plant-beneficial microorganisms allows insights into the arrangement of key genes which are

involved in biocontrol, plant health and growth promotion, as well as stress protection. In this study, a genome-based approach was conducted to optimize the performance of biocontrol agents (BCAs) towards *Rhizoctonia solani*. An antagonistic cocktail comprising of *Pseudomonas fluorescens* L13-6-12, *P. poae* RE*1-1-14, *Serratia plymuthica* 3Re4-18 was selected following an extended screening. Aspects influencing biological control of *R. solani,* such as sugar beet root colonization, plant growth promotion, and human safety were fortified [1, 2]. In long-term field trials performed over six consecutive years, *P. poae* RE*1-1-4 was proven to control late root rot caused by *R. solani*. The antagonistic cocktail applied to seeds showed positive effects by suppressing the fungal pathogen and increasing the number of healthy beets.

The genome of the endophyte *P. poae* RE*1-1-14 was completely sequenced and functionally analysed [3]. Key genes putatively important for biocontrol, plant promotion and general stress response by the production of antioxidants or detoxification have been identified in *P. poae* RE*1-1-14. Furthermore, genes were found which enable plant association and an endophytic lifestyle. Supporting microscopic analysis revealed the target colonization of *P. poae* RE*1-1-14 in the cortex of different sugar beet cultivars. Genome and corresponding transcriptomic analysis of the BCA represent a convenient way to improve and trigger processes which are involved in essential biocontrol mechanisms, e.g. for rhizosphere competence and antagonistic activities. To improve the biocontrol performance, essential genes and mechanisms will be identified.

[1] Zachow et al. 2010. Strain-specific colonisation pattern of *Rhizoctonia* antagonists in the root system of sugar beet. FEMS Microbiol Ecol 74:124-135.
[2] Zachow et al. 2009. *Caenorhabditis elegans* provides a valuable tool to evaluate the human pathogenic potential of bacterial biocontrol agents. Europ J Plant Pathol 125: 367-376.
[3] Müller et al. 2013 Complete genome sequence of the sugar beet endophyte *Pseudomonas poae* RE*1-1-14: a disease-suppressive bacterium. GenomeA 1(2):e00020-13. doi:10.1128/genomeA.00020-13.

P168

Ecology of *Lysobacter* species in natural disease suppressive soils

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Lysobacter species are Gram-negative bacteria that are widely distributed in diverse ecosystems, including soil, rhizosphere and freshwater habitats. Three closely related *Lysobacter* species (*Lysobacter antibioticus*, *Lysobacter capsici* and *Lysobacter gummosus*) were isolated from soils suppressive against *Rhizoctonia solani*, a devastating fungal pathogen of numerous economically important crops such as sugar beet, potato and rice. To date, the role of *Lysobacter* species in natural disease suppressive soils and the mechanisms involved in pathogen control remain largely unknown. Moreover, little is known about the population dynamics of these *Lysobacter* species in soil and rhizosphere environments. The overall goal of this study is to determine the abundance of these three *Lysobacter* species in soils suppressive against *Rhizoctonia solani*, and to study their distribution, population dynamics and intraspecific diversity. The abundance of the three *Lysobacter* species in the rhizosphere of sugar beet seedlings grown in soils with different level of suppressiveness against *Rhizoctonia solani* is determined by PhyloChip analysis and by a TaqMan detection method. The results of the PhyloChip analysis revealed that

the *Lysobacter* genus was more abundant in the suppressive soil than in conducive soil.. Subsequent *in vitro* bioassays showed that the three *Lysobacter* species inhibit the growth of *Rhizoctonia solani* as well as the growth of several other plant pathogenic fungi, oomycetes and bacteria.

Collectively these preliminary results suggest that *Lysobacter* species are more abundant in disease suppressive than in conducive soils and exhibit an antagonistic effect against *R. solani* and other plant pathogenic microorganisms. Current and future experiments are performed to study the population dynamics of *Lysobacter* species in disease suppressive soils and to determine if and how they suppress the fungal pathogen *R. solani*.

P169

Analysis of acetic acid bacterial population during industrial submerged red wine vinegar production based on 16S-23S rDNA ITS regions

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One of the most traditional products produced by acetic acid bacteria is vinegar. Despite long tradition, the industrial vinegar is still produced by a non-defined culture, so called seed vinegar from a previous oxidation cycle. Since 2002, an easy, quick and reliable identification of acetic acid bacteria is possible through comparison of restriction profiles of their PCR-amplified 16S-23S rDNA ITS regions to a database established on the reference strains (Trček and Teuber). We applied here this method for a systematic analysis of bacterial population from a well running oxidation cycle of an industrial process of vinegar production from red wine. At each of the eight sampling points ethanol and acetic acid concentrations were determined and bacterial strains isolated. The isolates were obtained after inoculation of RAE medium containing 1% of ethanol and 1% of acetic acid and after incubation in atmosphere with high humidity. The basic phenotypic analysis classified all isolates into the group of acetic acid bacteria. At the same time the bacterial biomass was harvested from each of the sampling points and directly analyzed for the presence of acetic acid bacteria. Results are showing that a single species of acetic acid bacteria from a group of *Gluconacetobacter intermedius/Gluconacetobacter oboediens* is dominating through the entire bioprocess. This result can be used as a marker for high percentage acetic acid producing microbiota in red wine vinegar bioreactor.

Trček J. and Teuber M. 2002. Genetic and restriction analysis of the 16S-23S rDNA internal transcribed spacer regions of the acetic acid bacteria. FEMS Microbiol Lett 208, 69-75.

P170

Genomic characterization of pilus-deficient derivatives of *Lactobacillus rhamnosus* GG

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Lactobacillus rhamnosus GG is one of the best characterized lactic acid bacteria and can be considered as a probiotic paradigm (1). Comparative genome analysis showed LGG to contain a genomic island encoding for pili previously found only in gram-positive pathogens (2, 3). Pili are proteinaceous appendages on the cell surface involved in host interaction and signaling (3). Architecture of the LGG pili has been studied extensively and found to consist of the major pilus protein SpaA, the minor pilus and putative stop-transfer protein SpaB and the tip protein SpaC that also decorates the pili (4). SpaC has the capacity to bind human mucus and is the major driver of the high mucus binding that has been associated with LGG (5). The genes for these structural pilus proteins are located in *spaCBA-srtC* operon that also encodes for sortase C, the enzyme that assembles the pili outside the cells (6).

In the present study we used a variety of induced and adaptive mutagenesis approaches to study the biosynthesis of the pili in LGG. While mutation selection has been used for decades to characterize biosynthetic pathways, the development of next generation sequencing methods allows for rapid characterization of the genomic impact of the mutations. Combination of these two powerful approaches has been applied to LGG for the selection of pilusless derivatives that were readily obtained by using several enriched population screens. The isolated mutants were characterized using dot blot analysis with anti-serum against the pili proteins. Relevant mutants were selected and their pilusless phenotype was confirmed using immuno-electron microscopy. Moreover, in mucus binding assays the mutants did not adhere to mucus and in adherence assays with Caco-2 and HT29 cells the binding was reduced to background levels while the parental LGG showed significant binding. Comparative genome sequence analysis using SOLiD and Illumina platforms was used to define the nature of the mutations in the obtained pilusless derivatives. These were found to include mutations in the *spaCBA-srtC* genes as well as other loci. The interpretation of these mutations and their effect on the pilus biosynthesis pathway will be presented.

1 Microb Cell Fact 2011; 10:S2
2 PNAS 2009; 106:17193
3 Nat Rev Microbiol 2011; 9:166
4 AEM 2012; 78:2337
5 AEM 2010; 76:2049
6 AEM 2013; 79:1923

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Cellulolytic bacteria from Steinhouse Lake in Antarctic Peninsula

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Organisms capable to survive on extreme environments have often evolved unique biosynthetic pathways to overcome all the adversities. However, despite all these challenges, life thrives in these environments with a remarkable microbial biodiversity. In the same way, the lakes of Antarctica are dominated by microbial communities. One important function refers to the enzymatic activity and enzymes responsible for carbon cycling are of particular importance in the maintenance of communities and ecosystems. The contribution of different groups of microorganisms to cellulose degradation in aquatic ecosystems remains obscure. The capacity of bacteria to utilize dissolved organic compounds at very low concentrations is important in oligotrophic lakes which have low concentration of available organic compounds. Thus, the main objective of this work was to access and evaluated the cellulolytic bacteria from Steinhouse Lake, located at Admiralty Bay, Antarctica. Thick cotton string served as cellulose bait for the isolation of bacteria. After 16 days of incubation, it was possible to observe biofilms and individual microorganisms on the surface of the cellulose baits. A total of 52 bacterial strains were recovered from the lake and tested for their cellulase activity and two of them, isolates CCMA 1184 and CCMA 1185, showed high cellulolytic activity. Phylogenetic analysis placed the isolates CCMA 1184 and CCMA 1185 into *Bacillus* 16S rRNA gene subclade closely related to *Bacillus subtilis* subsp. *subtilis* and *Bacillus safensis*, respectively. This study throws light on the presence of cellulolytic bacterial population in an Antarctic lake and its possible role in degradation of organic matter. Additionally, this was the first report on the cellulolytic bacterial at Steinhouse Lake.

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Antagonistic effects of the indigenous Greek isolate *Streptomyces rochei* ACTA1551, on *Fusarium oxysporum f.sp. lycopersici*

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Many studies have shown that several Greek ecosystems inhabit very interesting bacteria with biotechnological properties. Therefore *Streptomyces* isolates from diverse Greek habitats were selected for their antifungal activity against the common phytopathogenic fungi *Fusarium oxysporum*. The isolate encoded ACTA1551, phylogenetically relative to *Streptomyces rochei*, could strongly suppress the fungal growth when examined in antagonistic bioassays *in vitro*. *Streptomyces rochei* ACTA1551 was able to protect tomato seeds from *F. oxysporum* infection *in vivo* while it was shown to promote the growth of tomato plants when the pathogen was absent. In an initial effort towards the elucidation of the biochemical and physiological nature of ACTA1551 antifungal activity, extracts from solid streptomycete

cultures under antagonistic or/and not antagonistic conditions, were concentrated and fractionated. The metabolites involved to the antagonistic action of the isolate showed to be more than one and produced independently of the presence of the pathogen. The above observations could support the application of *Streptomyces rochei* ACTA1551 as biocontrol agent against *F. oxysporum*.

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The industrial use of coding sequences from metagenomic expression libraries – lessons learned from variance partitioning, genetic constraints and sampling probabilities

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Metagenomic expression libraries enable direct expression of environmental coding sequences (cds) in analyses of respective activities. Currently, there has been no development on models linking metagenomic primary sequences with functional properties. In order to fill this gap, (i) modeling of target finding in the metagenomic background was compiled incorporating as variables metagenome size, target gene copy number per organism, target gene length, fraction of organisms carrying target gene in community, community size, number and size of clones and other. (ii) Metagenomic clone libraries ((MCL); n>200) tapping heterogenous metagenome enzyme pool aiming at their commercial use was compiled. Redundancy analysis was used to identify whether the parameters used for construction of MCL or the nature of target enzymes explained the success in finding the desired functional activities. (iii) Direct clone libraries were retrieved and supplemented with the contextual and spatial data next to sequences from cultivated portion of microbial communities. Diversity analyses and variance partitioning were used to identify the key environmental parameters explaining distribution of target genes in the environment hence commanding sample selection. The probabilities of finding any target gene or the industrially relevant variant present in metagenomes of different size within MCL were modelled and agreed with the observed success rates. (iv) Functional characteristics expressed metagenomic pool was related to observed protein diversity in natural communities, genetic constraints on protein evolution and the price-performance ratio of the established industrially relevant enzyme applications obtained through directed protein evolution. Three orders of magnitude higher activities under 100-times higher substrate concentrations in industrial reaction conditions were present in minute trace fraction of all possible sequences. These traits are selected against by nature and consequently not present in the pool accessible to MCL. The need for a different strategy in searches for industrially relevant enzymes was exemplified by recent developments in guided evolution and also by the low pattenting rate from numerous publicly funded projects in coparison to the very few focused and private studies.

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Desert rhizosphere microbiota and plant resistance to water stress

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Introduction: Soil salinity and drought are among the most severe environmental stresses affecting plant growth and production around the world. While mycorrhiza have been widely studied for their contribute to plant resistance to water stress, few information are available about the role of bacteria.

Objectives: aim of the work was to study diversity and growth promotion potential of bacterial plant microbiome, evaluating its contribute in alleviating water stress.

Materials and methods: Two model plants, naturally subjected to water stress, were chosen: pepper (Capsicum annuum L.), a drought-sensitive crop cultivated in traditional Egyptian farm and Salicornia (S. strobilacea), a wild halophyte plant growing in Tunisian hypersaline soils (Chott). DGGE fingerprinting was used to describe structure and diversity of rhizosphere and endosphere bacterial communities, in comparison with plant-free soils. Wide collections of bacterial isolates were established from the two plants, and the strains were screened in vitro and in vivo for plant growth promotion (PGP) activities and for the resistance to environmental stresses typical of desert environments, i.e. temperature, salinity and osmotic pressure.

Results: The results showed that the plant determined a selective pressure on the phylogenetic diversity of the associated populations. The screening of the bacterial collection showed that a significant fraction of the cultivable bacterial populations associated to desert plants display multiple PGP activities and are adapted to survive under severe environmental conditions. In vivo experiments with selected strains demonstrated, moreover, the capacity of PGP rhizobacteria to colonize the root surface and to enhance plant photosynthetic activity and biomass synthesis (up to 40%) under drought stress.

Conclusions: in the sight of "reverse desertification", crop cultivation in arid, saline and degraded lands is a key practice for the preservation of soil stability and fertility, with the root system acting as a "resource island" able to attract and select microbial communities endowed with multiple PGP traits that sustain plant development under water stress conditions.

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Sensitivity of CH_4- and CO_2-producing microbial communities in anoxic peat to changing environment in a peatland transplantation experiment

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Anoxic peat of northern peatlands stores large amounts of carbon, which is released as methane (CH_4) and carbon dioxide (CO_2) through microbial activity. Peatlands differing in vegetation, water chemistry and pH show variation of CH_4 emission but also

in the ratio of CH_4 and CO_2 produced anaerobically. Nutrient-poor acidic bogs produce far more CO_2 than CH_4, whereas in groundwater-fed fens CH_4 production is more dominant. Changes of CH_4/CO_2 ratio affect the strength of the peatland as a greenhouse gas source.

To address the sensitivity of CH_4/CO_2 ratio and anaerobic microbes producing these gases to environmental conditions, we carried out a reciprocal field transplantation experiment. Peat was enclosed in membrane bags with pore size of 0.2 µm, allowing movement of water and soluble compounds but preventing microbial colonization of the transplanted peat from the outside. Transplantations were made from bog to fen, fen to bog, and as controls within bog and fen. The membrane bags were placed in peat below the water level and collected after two months for analysis of CH_4 and CO_2 production and microbial communities. RNA was extracted from peat for comparison of bacterial communities by 16S rRNA T-RFLP and methanogen communities by *mcrA* T-RFLP. Abundance of bacteria and methanogens will be analyzed by quantitative PCR.

Methane production of bog peat increased with transplantation almost to the level of the native production of fen peat. Conversely, fen peat transplanted to the bog produced significantly less CH_4 and even less than initial bog peat. Anaerobic CO_2 production increased slightly with transplantation from bog to fen, suggesting an increase in overall microbial activity in more favourable conditions, but no effect was seen with transplantation from fen to bog. The results so far suggest that sensitivity of methanogenesis to conditions in bogs, such as low pH, drives differences in CH_4/CO_2 ratio in peatlands.

P176
Bet-hedging as a fitness character in *Paracoccus denitrificans* during transitions from aerobic respiration to denitrification

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Introduction: Bacteria with a respiratory energy metabolism face a regulatory challenge in an environment which fluctuates with respect to oxygen availability. On the one hand they should optimize the efficiency with which they respire oxygen, on the other they need to sense when a switch to alternative electron acceptors is necessary in order to sustain growth.

Objective: We set out to test our 0 hypothesis: when aerobically respiring cultures of *Paracoccus denitrificans* or *Thauera linaloolentis* face imminent anoxia in the presence of nitrate or nitrite, the entire populations switch to denitrification.

Materials & Methods: Oxygen consumption and accumulation of gaseous NO_x in cultures growing in NO_3^-/NO_2^- supplemented liquid medium were monitored by frequent headspace sampling during transitions from oxic to anoxic conditions. To do this, we employed a semi-automatic incubation system consisting of a thermostatic water bath holding 15 stirred vials and an autosampler connected to a Varian CP 4700 micro-GC with a 10 m poraPLOT U and a 20 m Mol Sieve 5 A column (in parallel) each equipped with a thermal conductivity detector (TCD) and a chemoluminescence NO_x analyser (Model 200A, Advanced Pollution Instrumentation, USA). Guided by gas measurements, liquid sampling was undertaken for additional analyses such as quantification of $[NO_2^-]$ and mRNA. Findings were corroborated by microscopy of nalidixic acid treated cultures as well as plate counts.

Results: In *P. denitrificans* there is strong evidence for a bet-hedging strategy where, as O_2 approaches depletion, only a minor fraction (F_{den}) of the culture switches to denitrification. This is demonstrated by a dramatic decrease in total e^-- flow at O_2 exhaustion, followed by balanced growth throughout the anoxic phase. In *P. denitrificans*, F_{den} is typically below 10% in cultures with 1% (v/v) initial O_2. A contrast is found in *T. linaloolentis*, whose denitrification profile is characterized by a rapid onset of NO_x reduction and lack of a discernible drop in e^-- flow upon O_2 depletion. This suggests a 100% switch to denitrification. Additional estimations of F_{den} employing microscopy and plating techniques strongly supported these findings.

Conclusion: F_{den} can be seen as a fitness character. While a 100% switch is rewarding if anoxia lasts long, the bet-hedging strategy adopted by *P. denitrificans* is likely to be favorable if anoxia is frequent but transient.

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Ecophysiology of *Vibrio harveyi* and *Vibrio ruber* in viscous media

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Bacterial cells in planktonic and biofilm state greatly differ in their physiology, most likely due to altered molecular diffusion and transport processes as a consequence of changed viscosity. There is, however, a lack of knowledge about bacterial ecophysiology in environments with intermediary viscosity (i.e. human mucus, sediments and viscous activated sludge). In this study physiology of *Vibrio harveyi* and *Vibrio ruber* was determined in liquid M9 medium supplied with hydroxyethyl cellulose, which changed the growth medium viscosity in the range from 0.8 to 30 mPas.

Both strains exhibited similar growth and metabolic activity pattern at tested viscosities. At viscosities higher than 2.4 mPas cells had to increase their total metabolic activity in order to keep up with the nutrient demand and maintain the same growth rate. Above 2.4 mPas the pentose phosphate pathway activity was notably increased, indicating an enlarged need for biosynthetic intermediates and reducing equivalents. These were not used for growth or morphological changes (i.e. cell shape and flagellation type remained the same). Furthermore, *V. ruber*, which is capable of producing prodigiosin as a secondary metabolite, decreased its pigmentation at 8 mPas or higher. Besides pigmentation, cell motility, which is important for obtaining nutrients, also requires a substantial amount of energy. Surprisingly, comparison of *V. harveyi* WT and a non-motile mutant showed that motility was beneficial only at viscosities up to 7.5 mPas, since the mutant had significantly lower growth efficiency than the WT. The loss of the WT strains advantage at viscosities higher than 7.5 mPas, implies that there are other processes employed in the adaptation to viscous media.

This is one of the few studies addressing the effect of environmental viscosity on bacterial physiology. Obtained results

suggest that viscosity may considerably affect the bacterial primary and secondary metabolism of *Vibrio* species and should therefore be included as a parameter in ecophysiological studies.

P178
Differential molecular-based monitoring of the filamentous growth of *Sphaerotilus natans*

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Introduction: Activated sludge is the most widespread biological wastewater treatment technology used over the world. However, the main problem of this process is due to the invasive proliferation of filamentous bacteria.

Sphaerotilus natans is a model bacterium involved in certain cases of bulking. This bacterium is able to grow as planktonic cells or as sheathed filaments. Conventional microbiological methods are problematic under filamentous growth and PCR techniques can only quantify the total cell amount.

Objectives: The aim of this work is to develop a method to quantify bulking-involved bacteria under their two growing morphologies: filamentous and planktonic, with the purpose of evaluating the factors inducting filamentation, such as nutrient availability.

Materials & methods: A set of qPCR primers specifics to *S. natans* has been designed, to be used in pure cultures as well as in complex matrices (i.e. activated sludge). These primers are targeted against the gene *sthA*. qPCR has been used to quantify cells from global culture samples as well as 3 µm-pore size polycarbonate filters and filtrates, in which filaments and planktonic cells are respectively the sole cell form. The different fractions were observed in scanning electron microscopy through the filtration process to validate its efficiency.

Results: The set of designed qPCR primers reveals high specificity to *S. natans*. It amplifies the strains ATCC 15291, 13338 and 13929 but not *Leptothrix* strains, which are phylogenetically close, or strain 29330, which has recently been reassigned as *S. hippei*.

The 3 µm filtration allows effective separation of both growth forms, and filament free suspensions of planktonic cells are obtained.

This method has been validated by monitoring the effect of nutrient-limited media on *S. natans* cultures. Its filamentous induction effect is clearly reflected by the growth kinetics obtained.

Conclusion: This powerful method allows quantifying the effect of factors inducing bacterial filamentation and therefore it may shed light on bulking and other environmental processes where filamentous growth is crucial.

P179
Exopolymer diversity and the role of levan in Bacillus subtilis biofilms

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Exopolymeric substances (EPS) are important for biofilm formation and their chemical composition may influence the biofilm properties. These relationships are not well understood in *B. subtilis* biofilms, which can also form on plant roots. Plant exudates may be rich in sucrose, which is a precursor of a polysaccharide, levan. In our work, chemical composition of EPS from *Bacillus subtilis* NCIB 3610 biofilms grown in sucrose-rich (SYM) and sucrose poor (MSgg and Czapek) media was studied to address the role of levan in biofilm formation. We observed marked differences in composition of EPS polymers isolated from all three biofilms or from spent media below the biofilms. The polysaccharide levan dominated the EPS of SYM grown biofilms, while EPS from biofilms grown in sucrose poor media contained significant amounts of proteins and DNA in addition to polysaccharides. The EPS polymers differed also in size with very large polymers (Mw > 2000 kDa) found only in biofilms, while small polymers (Mw < 200 kD) dominated in the EPS isolated from spent media. In terms of biofilm thickness the *eps* knockout was significant and dominant over the *tasA* knockout in all media. The biofilm defective phenotypes of *tasA* and *eps* mutants were however, partially compensated in the sucrose rich SYM medium. The sucrose supplementation of Czapek and MSgg media increased the thickness and stability of biofilms compared to non-supplemented controls. Since sucrose is essential for synthesis of levan and the presence of levan was confirmed in all biofilms grown in media containing sucrose, this study for the first time shows that levan, although not essential for biofilm formation, can be a structural and possibly stabilizing component of *B. subtilis* floating biofilms. In addition, we propose that this polysaccharide, when incorporated into the biofilm EPS, may also serve as a nutritional reserve.

P180
Prodigiosin – mechanisms of antimicrobial action

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Bacterial pigments known as prodigiosins are important secondary metabolites showing antimicrobial, cytotoxic, anticancer and immunosuppressive activities. Prodigiosin isolated from red pigmented marine bacterium *Vibrio ruber* DSM 14379 exhibits potent antimicrobial activity against a broad range of bacteria, including both Gram positive and Gram negative bacteria. Despite prodigiosin is a well studied molecule, the mechanism of its antimicrobial action remains unknown. Therefore, structure and function of *Vibrio harveyi*, *Bacillus* sp. and *Escherichia coli* were studied after prodigiosin treatment.

Co-culturing of these bacterial strains with prodigiosin producing bacterium *Vibrio ruber* resulted in significant inhibition of all strains showing lower Malthusian fitness if compared to growth in monoculture. Treating *Bacillus* sp. with isolated prodigiosin resulted in cell lysis and morphological changes followed by a

major decay in optical density of *Bacillus* sp. culture. Similar response to prodigiosin treatment was observed in *V. harveyi*. Moreover, some other parameters including bacterial structure and function were tested. The results showed that there was no DNA degradation and there was a negative effect on cell membrane integrity after treating cells with prodigiosin. On the other hand, growth of *E. coli* was only inhibited by prodigiosin. Results suggest that in absence of significant DNA damage and changes in cell membranes, metabolic activity of cells was impaired after prodigiosin treatment.

Obtained results suggest that prodigiosin acts bacteriostatically on *E. coli* and as bactericidal agent on *V. harveyi* and *Bacillus* sp. MIC and MBC values confirmed these findings. This study shows that prodigiosin inhibits bacteria non-related to *V. ruber* with different mechanisms.

P181

Amazonian resistome: evaluating antibiotic resistance abundance and diversity across a French Guiana forest soil gradient through metagenomic approaches

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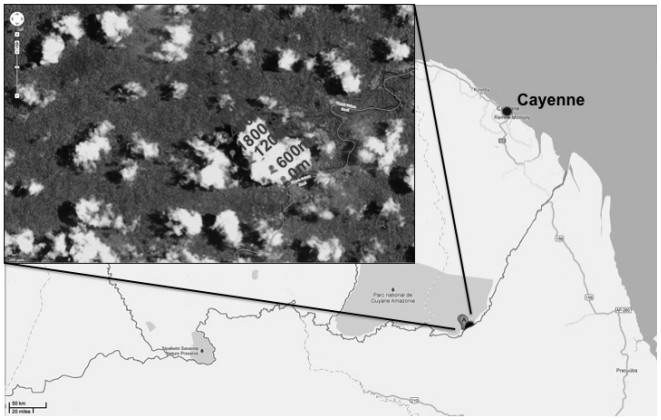
Antibiotic resistance gene determinants acquired by pathogenic bacteria are a serious healthcare issue. While this antibiotic resistance phenomenon is well studied in the clinical context, little information concerning the *environmental resistome* is currently available. However, a number of studies have reported the isolation of resistant bacteria from aquatic and soil samples. A better understanding of antibiotic resistance prevalence and diversity in the environment will help elucidate resistant gene movement between environmental and clinical pathogenic bacteria. Therefore, we focused on a well-preserved study site, the Trois-Sauts village located in Guiana Amazonian National Park to which access is restricted to permanent residents since 1970. Trois-Sauts is isolated from any sources of antibiotics except those provided by the village dispensary, which registers every prescription. A 3000m soil transect was sampled in triplicates every 600m resulting in 18 soil samples from the village to the forest (figure 1). We hypothesized that a single antibiotic source could affect the soil bacterial community in terms of antibiotic resistance genes and mobile genetic element (MGEs) abundance. Soil metagenomic DNA was extracted from all samples and submitted directly to Roche 454 pyrosequencing, resulting in 18 datasets of metagenomic DNA sequences reads. Each dataset was annotated independently and antibiotic resistance genes were screened by sequence homology to a reference database. We also performed quantitative PCR on the same DNA samples, focusing on some antibiotic resistance representative marker genes (such as bla_{SHV}, bla_{TEM}, sul(I), tet(G), tet(H), cfiA) already found in environmental samples. Mobile genetic element markers (such as int1, int2 for integrons and tra, korB and rep for conjugative plasmids) were also quantified. Correlations between the presence of mobile genetic elements and antibiotic resistance genes could be established. However, we did not observe a significant increase in antibiotic resistance gene load between distal (3000m) and village (0m) samples.



P182

Potential biodegradability of diesel oil by _E. coli_ strain caring the _alk_B gene from the soil metagenome

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Bacteria are the main agents of degradation of petroleum contaminants and the gene *alk*B encoding the enzyme alkane hydroxylase is responsible for the degradation of hydrocarbons. Currently in Brazil the B5 fuel which is composed of 95% of diesel oil and 5% biodiesel, is used. It contains a mixture of alkanes, alkenes and aromatic compounds. The soil used for obtaining the consortium was collected from the site contaminated by the factory that manufactured automotive lubricants site in Ribeirão Preto County, São Paulo State (Brazil) (at 21 ° 06 ' S 47 ° 49¢). The consortium was harvested after seven days of incubation and used for construction of a metagenomic library in the fosmid vector pCC2FOS™. We have been customised nylon macroarrays containing fluorescent probes and the DNA, from the genes hybridized followed the experiments. The genes *alk*B from the metagenomic library was sequenced and submitted to NCBI / BLAST and was chosen to be an identified undescribed species according to the similarity which showed 86% similarity to *alk*B gene from *Pseudomonas aeruginosa*, to be considered the same species the percentage is above 98% similarity. PCR primers targeting the *alkB* gene in the metagenomic library were designed. The *alk*B gene was amplified and sub cloned into the expression vector pET 28a using *Escherichia coli* BL21 (DE3) as the host strain. The clone was tested for its potential to degrade diesel oil 5% (v / v) in defined minimal medium (MM). Cultures were incubated for 21 days in small scale erlenmeyers and exposed to rotation for 60 seconds every 24 hours. An increase in OD_{600} was observed only in the experimental variant B. (Fig.1). Biodegradability was confirmed using falcon tubes under steady rotation: (1) MM + diesel oil (2) MM + clone *alk*B + diesel oil, (3) MM + clone *alk*B. After 21 days of cultivation the culture was inoculated on Luria Bertani (LB) agar plates containing kanamycin and colonies were observed only when inoculated from the experimental variant B (Fig. 2) suggesting that the diesel oil was consumed as the carbon source. The results suggest that the clone carrying *alk*B has a potential for biodegradation of hydrocarbons.

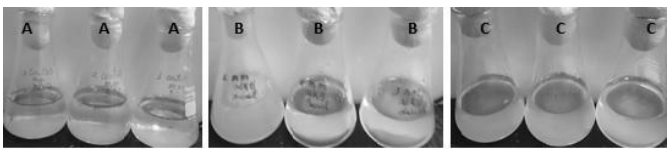

Figure 1: Experiments monitored during 21 days of cultivation the *E. coli* carrying the *alk*B gene under occasional rotation: (A) MM + diesel oil, (B) MM + diesel oil + clone *alk*B, and (C) MM + clone *alk*B.

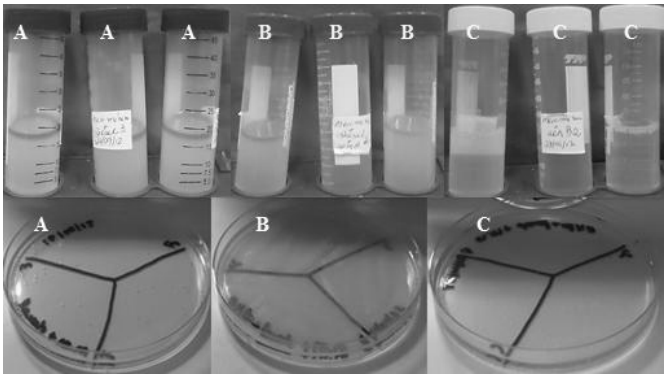

Figure 2: Experiments monitored during 21 days under steady rotation: (A) MM + diesel oil, (B) MM + diesel oil + clone a*lk*B, and (C) MM + clone *alk*B. After 21 days of cultivation the *E. coli* carrying the *alk*B gene was inoculated the same in LB agar plates respectively.

Keywords: microorganism soil, hydrocarbons, bioconversion. Acknowledgements:We thank Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for the financial support.

P183
Response of the soil microbial community to application of manure from difloxacin-treated pigs

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Difloxacin (DIF) belongs to the class of fluoroquinolone antibiotics which have been intensively used for the treatment of bacterial infections in veterinary and human medicine. Recently it was shown that after oral application to pigs, only a minor part of DIF is metabolized by the animal and the main part of the parent compound is excreted in bioactive concentrations.

The aim of this field study was to assess the influence of manure from DIF-treated pigs compared to manure from unmedicated pigs on the bacterial community structure and resistance gene abundance in bulk soil and rhizosphere of maize. The manures were applied in amounts of 30 m^3 ha^{-1} on experimental plots (3 x 6 m, n=4) and incorporated to a depth of 12 cm, followed by sowing of maize (*Zea mays* L.). Soil samples were taken on days 0, 7, 14, 28, 71, 105 and 140 after manure application. A significant effect of DIF manure on the bacterial community composition in bulk soil was revealed by denaturing gradient gel electrophoresis (DGGE) of bacterial 16S rRNA gene fragments amplified from total community DNA. Quinolone resistance genes *qnrB* and *qnrS1/S2* were detected by PCR and subsequent hybridization while *qnrA* was not detected. Quantitative real-time PCR revealed that the abundance of integrase genes *intI1* of class I integrons and sulfonamide resistance genes *sul1* and *sul2* were increased in DIF manure treated bulk soil and rhizosphere, relative to 16S rRNA genes, while *traN* genes specific for LowGC-type plasmids, which in recent years were assumed to play an important role in conferring sulfadiazine resistance in manure treated soils, were increased in bulk soil. These effects of DIF manure on the soil microbial community might be attributed to a selective pressure exerted by DIF in soil and/or to resistant commensal bacteria selected in the pig gut by administration of DIF.

In conclusion, although DIF is reported to be hardly accessible to the soil microbial community due to the rapid formation of non-extractable residues, manure from DIF-treated pigs can significantly alter the soil microbial community and the abundance of resistance genes and mobile genetic elements over a period of 140 days compared to manure from untreated pigs which has to be considered in further assessments of risks associated with the spread of resistance genes in the environment.

P184
Impact of amendment with raw organic wastes on the prevalence of human pathogens and antibiotic resistance spread in agricultural fields

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Question: Increase in human population and human activities lead to an increase in waste production. Recycling organic wastes in agriculture can improve soil quality, favour plant growth or suppress diseases caused by soil borne pathogens. Occidental countries prohibit final deposition of waste in landfills without prior treatment. However such practices are not yet worldwide used and untreated waste addition to cropped plots could be at risk for human health. Here we report on the impact of raw urban waste amendment on emergence and / or spread of pathogens and antibiotic resistance genes in agricultural land in Burkina Faso.

Methods: Non amended and amended soils were sampled at three sites in the periphery of Ouagadougou in 2008 and 2011. We enumerated heterotrophic cultivable microflora, faecal indicator bacteria and human pathogenic species and determined antibiotic susceptibilities of opportunistic pathogen isolates. The impact on antibiotic resistance genes focused on the distribution and diversity of various β-lactamase-encoding genes.

Results: An enrichment in total bacteria in amended plots was observed. No pathogens such as enterococci, fecal coliform, *Staphylococcus aureus* were detected and the rare detection of the opportunistic human pathogen *Pseudomonas aeruginosa* suggested that the amendments were not sources of these pathogens. However, monitoring populations of *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia*, opportunistic pathogens of man commonly found in soils, showed the

enrichment of these populations in amended plots. Antibiotic susceptibility test evidenced a high level of multi-resistance towards antibiotics. DNA extracts from amended soils also led to the detection of bla_{OXA-10}-type, and bla_{SHV}. On the opposite bla_{CTX-M}-like genes, bla_{VIM}, bla_{TEM} and bla_{IMP} were not detected.

Conclusion: This work showed that waste dispersion on agricultural land may pose health risks. Composting process can be used to ensure microbial safety and prevent development of food-borne illness pathogen and standard limits have been defined for several pathogens and bio-indicators. However these standards did not yet take into account human opportunistic pathogens and/or antibiotic resistant bacterial species potentially present in the original waste or developing during the composting process.

P185

Perspectives on combinatorial biological, chemical and physical parameters in soil quality: an approach for sugarcane production systems

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Changes in soil microbial communities across space are often correlated with the differences in soil chemistry and physics. As an example, differential responses of the Acidobacteria subgroups to specific chemical soil factors were found to agricultural management of soybean in Amazon forest soils. A better understanding of how agricultural management affects the soil-borne microbial communities that drive numerous terrestrial ecosystem services will support the development of more productive, sustainable agroecosystems. Collectively, these ecosystem services have been defined as soil quality. After almost four decades of soil microbial biomass (MB) studies in Brazilian ecosystems, this approach is one of the most used as indicator of soil quality because the MB responds promptly to environmental changes, often much earlier than physical and chemical parameters. Additionally, some important parameters derived from MB, such as the ratio of MB carbon (MB-C) to the total soil organic carbon (TSOC), and the metabolic quotient (qCO_2), which is the ratio of basal respiration (BR) to the total MB-C, have been used to indicate the soil vulnerability to disturbance in terms of resilience and resistance. However, at present, we can develop biologically relevant methods to assess soil quality based on chemistry, physics and high-throughput biological screening applied to the bio-indicators discovery. In this sense, we propose use high-throughput DNA sequencing technology for screening taxonomic and functional microbial bio-indicators in soils under sugarcane biomass production systems and associate them to soil characteristics and processes occurring in soil, such as, fertility, nutrients availability, microbial biomass, enzyme activity and N_2O, CO_2 and CH_4 emissions from soil. This combinatorial approach has been initially applied to evaluate the potential impacts of bio-based sugarcane production processes on microbial functions and soil quality. [FAPESP, BE-BASIC, CNPq]

P186

Examining Acidobacteria as bio-indicators in Amazon soils – an approach using landscape-scale

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The phylum Acidobacteria dominates in low pH soils of the tropics. Recent results from soils sampled in the Southeastern Brazilian Amazon have opened the possibilities to explore acidobacterial subgroups as early-warning soil bio-indicators in the Amazon area. Here we examine the abundance of Acidobacteria as potential bio-indicators for soil disturbance at the landscape level in Amazonia. Members of the Acidobacteria were detected in soil samples by high-thoughput sequencing (454 pyrotag) of the bacterial 16S rRNA gene along a gradient in landscape management intensity. The gradient spanned from adjacent primary forests (PF), to pastures (PA), and secondary forest (SF) sites located in the Brazilian Amazon. At each site, soils were sampled using a spatial nested sampling scheme centered on a 100-m^2 quadrat, with 10-m^2, 1-m^2, 0.1-m^2, and 0.01-m^2 quadrats nested within, for a total of 12 sampling points per 100-m^2 quadrat. Numerically, Acidobacteria represented 20% of the bacterial 16S rRNA gene sequences from PF soils (30,191 sequences), 11% from PA soils (64,503 sequences), and 24% from SF soil (13,648 sequences). Relative abundance of Acidobacteria subdivisions was correlated with soil factors, such as pH, C, N, C:N ratio, P, S, K, Ca, Mg, Al, base and Al saturation, cation-exchange capacity and moisture content. Significant correlations to soil chemical characteristics emerged for all soils, with Acidobacteria subgroup 1 dominating all sites. Subgroups 4, 6, 7, 10, 17, and 25 were significantly correlated to soil characteristics from PA. Subgroups 2, 3, 5, 9, 11, and 13 were statistically associated to PF soils characteristics. SC site was composed almost exclusively by members of subgroup 1. These results obtained based on high-thoughput sequencing from 132 different soil samples expands the possibilities to explore acidobacterial subgroups as early-warning soil bio-indicators in the Amazon area. [USDA, FAPESP, CNPq]

P187

Chemically-enhanced microbial degradation of recalcitrant chlorinated compounds

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Many of persistent organic pollutants (POPs) addressed by the Convention of Stockholm are chlorinated compounds (such as lindane, mirex or kepone). While several factors (such as bioavailability) can play important roles in blocking biodegradation, often the first step of the degradation pathway is

rate limiting. Thus, while the degradation might be thermodynamically favorable, the slow kinetics can inhibit our exploration of possible biodegradation pathways and the effect of degradation on the microbial community of the impacted ecosystem. The approach used here was to apply a range of different chemical conditions (oxidizing and reducing) in order to favor the biodegradation of a range of POPs (lindane, mirex, TCE, Kepone). A range of metalic salts with different standard potentials were added to polluted soil incubations. Biodegradation was monitored by gas chromatography/mass spectrometry. The metallic salts acted as catalysers for degradation of chlorinated molecules, and thus, affected the potential biodegradation mediated by the soil bacterial community. The changes in the microbial community were monitored by both RISA fingerprinting and phylogenetic microarray. These results were correlated with the biodegradation rates and metabolites in order to identify potential pollutant degraders.

P188

Draft genome of a MCPA-degrading *Sphingomonas* reveals unique genetic context of the less-studied *cadAB* genes in a transposon situated on a conjugative plasmid

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The MCPA-degrader *Sphingomonas* sp. ERG5 was previously isolated from an arable soil, using a novel technique for isolation of oligotrophs. It was observed with PCR that this strain contains the gene *cadA*, encoding the large subunit of a 2,4-D oxygenase. This gene is not very well characterized, but transcript and regulatory studies will be performed in the future, using *Sphingomonas* sp. ERG5.

The first step in further characterization of the *cadA* gene was to investigate the genetic context of this gene.

Using Illumina-sequencing, the genome of *Sphingomonas* sp. ERG5 was sequenced with a 90x coverage, revealing a ~106kbp conjugative plasmid containing a ~32.5kbp transposon. Within the transposon, genes were present encoding the complete degradative pathway of 2,4-D and MCPA, including regulatory and uptake genes. The classical *tfdA*α-gene, encoding the first step in degradation of 2,4-D and related compounds, of group III 2,4-D degraders was not present in the draft genome. However, the alternative and less-studied *cadAB* genes, also providing the initial degradation step, were found in the transposon, along with the other 2,4-D-degradation-associated genes, *tfdBCDEFKR* and *cadR*. The transposon was confirmed to be located on the plasmid with PCR and amplicon sequencing.

This study presents the hitherto deepest molecular investigation of the genetic context of *cadAB*. Part of the transposon contains sequence displaying high homology to previously analyzed 2,4-D degradation genes isolated from various geographical regions, suggesting very rapid dispersal and high conservation of the genes involved in chlorinated phenoxy acid herbicide degradation within the *Sphingomonas* genus.

P189

Detection of antibiotic resistance genes and mobile genetic elements in fermentation residues from biogas plants and manures from different pig producing facilities

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In livestock breeding animals are treated extensively with antibiotics (ABs) which they excrete unchanged or as metabolites. These ABs may select for resistant bacteria in the intestine of the animals and in soil to which manures or fermentation residues are applied as fertilizers. This might lead to an enrichment of resistant bacteria and antibiotic resistance genes (ARGs) in the microbial community. Via mobile genetic elements (MGEs) these genes may spread even among phylogenetically distant bacteria and new resistance phenotypes of pathogens might occur.

The aim of our project is to monitor ARGs and MGEs in manures and fermentation residues of biogas plants in order to develop mitigation strategies.

We sampled 16 different pig producing facilities and 8 biogas plants (fermenters fed with pig manure, one alternatively fed with bovine manure) at different processing steps. The total community (TC) DNA was analyzed for the presence of ARGs and MGEs (via PCR, Southern blot and quantitative real time PCR). Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments amplified from TC-DNA was used to analyze the composition of bacterial communities in manures from different farms and during fermentation in biogas plants.

We could detect several MGEs (plasmids, integrons) and all ARGs tested (*sul1*, *sul2*, *sul3*, *tetA*, *aadA1*) not only in manures but also in the biogas residues. A decrease in the abundance of *sul1* and *intI1* relative to 16S rRNA genes during fermentation and a slight increase after storage of residues was observed in all biogas plants. Changes in the composition of bacterial communities during the different steps in biogas plants were revealed by DGGE.

Our data indicate that a reduction of ARGs and MGEs was not achieved in a mesophilic fermentation process in biogas plants and thus both piggery manures and fermentation residues used as fertilizer in the agro-ecosystem might contribute to the spread of ARGs and MGEs.

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P190

Management of arable soil to reduce eutrophication in changing climate – how no-till affects microbial communities and individual phylotypes

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Climate change predicts new challenges for agriculture in the Northern Hemisphere, such as increased erosion and nutrient run off as a result of milder winters. Plant cover during this season could reduce run offs. No till, leaving plant residues after harvest, is increasingly favored for arable land management in Europe. Detailed microbial community data of soil gives room for the use of soil ecosystem services and may offer an insight to how soil micro-organisms respond to agricultural management (Sipila et al. AEM, 2013). Bacterial community structures were resolved in a cross site study of six arable fields with no-till and till treatment in depth 0-5, 5-10 and 10-20 cm. Bacterial 16S rRNA tags were sequenced using 454 FLX Titanium pyrosequencing producing 195 977 16S rRNA tags. The main bacterial phyla in arable soil were Acidobacteria (24%), Proteobacteria (22 %) and Actinobacteria (19%). Robust pair wise analysis revealed that tillage treatment changed the bacterial phyla, genus and OTU abundances. Concurrent effects of treatment and depth on bacterial communities were demonstrated in cross site setup in spite of high variation between fields. Multifactorial analysis linked bacterial phyla to soil parameters. The abundance of Gemmatimonadetes, Actinobacteria and Chloroflexi was higher in soil with high clay, total C content and water holding capacity. The presented data set enables predictions of how long term no-till management change arable soil bacterial communities on regional level.

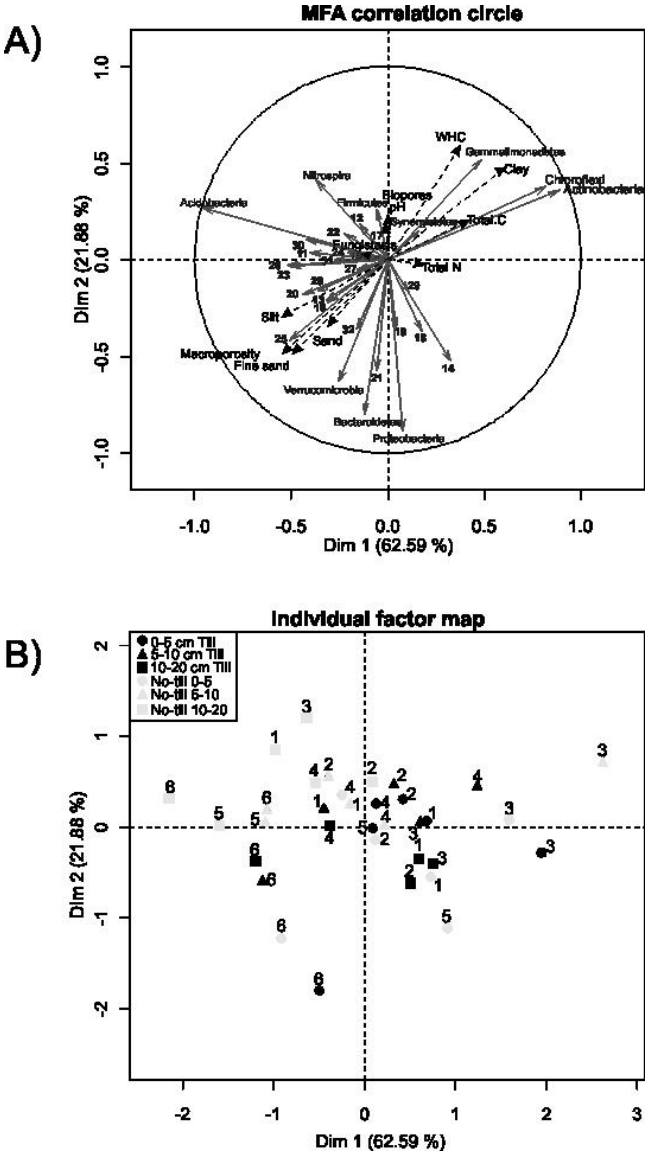

Figure 1. Multifactorial analysis (MFA) of bacterial phyla distribution and environmental variables in arable soil. The centered phyla distribution as factor 1, scaled environmental variables as factor 2 and treatments (depth and tillage intensity) as categorical variables. A) Correlation circle, ten most abundant phylum are marked. Rest of the phyla are coded with numbers 11:Planctomycetes, 12:OP10, 13:Bacteria incertae sedis, 14:Cyanobacteria, 15:Aquificae, 16:Chrysiogenetes, 17:Deferribacteres, 18:Crenarchaeota, 19:Tenericutes, 20:Deinococcus-Thermus, 21:TM7, 22:Caldiserica, 23:Thermotogae, 24:Thermodesulfobacteria, 25:Spirochaetes, 26:Dictyoglomi, 27:Chlamydiae, 28:OD1,29:Lentisphaerae, 30:OP11,31:Euryarchaeota, 32:Fusobacteria B) MFA analysis of individual soil samples.

P191

Response of methane cycling communities to the succession of re-vegetated cut-away peatlands

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Peat extraction is destructive for peatland ecosystems. In Finland, peat is currently excavated on nearly 70 000 ha, mostly for energy use. One of the ways to minimize the negative effects of peat mining is to restore cut-away areas close to their natural, water-saturated state. Based on gas emission measurements, abandoned cut-away peatlands are usually net carbon sources but either restoration- or self-induced recolonization of peatland vegetation may turn them back to carbon sinks. Methane (CH_4) emissions start to slowly rise due to increased plant cover, resembling the natural peatland carbon cycling. Despite of the importance of microbes for the carbon cycle, the knowledge of re-vegetation induced changes on associated microbial communities is severely lacking.

We studied the succession patterns of methanotrophic bacteria (MOB) and methanogenic archaea on three abandoned cut-away sites representing different stages of re-vegetation process (2, 17 and 63 years past abandonment) on the oldest peat mining area of Finland, Aitoneva (62◦12'N, 23◦18'E). In addition to peat, living *Sphagnum* mosses were sampled, as they are known to act as CH_4 biofilters between the ecosystem and the atmosphere. Compared to the pristine control fen, CH_4 oxidation potential of the peat was on a similar level already after 17 years. Type II MOB were over an order of magnitude more abundant on 63 y and pristine sites than on the younger sites and, based on a *pmoA*-microarray, more diverse than type I MOB on all of the sites. In *Sphagnum* mosses CH_4 oxidation was on a similar scale on all sites but the amount of the dominant type II MOB increased with restoration succession. MOB communities in the peat and in the mosses were most similar on the 63 y and the pristine sites and differed most on the 2 y site, which also had the highest within site variation in MOB community compositions. Methanogenic abundance (*mcrA* copies) and potential activity were clearly higher in the peat of 17 y and 63 y sites than on the youngest 2 y site although still lower than on the pristine fen. T-RFLP analysis of the *mcrA* gene is in progress.

Further analysis aims to link microbial variables to vegetation, pH and hydrology data. Based on the results so far, microbes associated with the CH_4 cycling seem to react relatively fast to the rewetting and re-vegetation of cut-away areas.

P192

Linking soil functioning with functional microbial diversity in agricultural soils – the normal operating range of arginine degradation across The Netherlands

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Question: A reliable risk assessment of possible adverse effects of soil stressors depends on knowledge of the basic variations in soil functions and microbial diversity. Such a "normal operating range" (baseline) can be used to understand the relevance of potential changes induced in the soils (*e.g.* by genetically modified plants or other applied stresses). We studied the baseline of the transformation of low-molecular weight carbon compounds by soil microorganisms and investigated the possible link between the structure of an active microbial community and the variability in soil functioning (carbon degradation) in normal conditions and after application of a strong disturbance.

Methods: Eight agricultural soils with different characteristics were chosen across The Netherlands and sampled in four seasons. To investigate carbon transformation, respiration kinetics of oxygen consumption during decomposition of a root exudate model compound (arginine) were measured in each season and also after application of a temperature and humidity stress. A stable isotope probing approach was used for studying the active bacterial degraders. Their diversity was determined in ^{13}C-labelled DNA by T-RFLP.

Results: In all investigated soils in normal condition, we observed relatively similar profiles of the arginine degrading bacterial communities, despite different soils characteristics. The composition of the degrader communities was correlated mainly with pH and organic matter content differences, and the active communities were dominated by the genera *Pseudomonas* and *Arthrobacter*. The kinetic respiration parameters, such as the respiration rate, were not significantly correlated with the community composition. The application of a strong soil disturbance shifted the kinetic parameters of some soils out of the normal operating range.

Conclusions: We observed that the degrading communities can be relatively stable at normal conditions. The community composition did not explain differences in degradation of arginine, pointing to the fact that other parameters (such as size of the degrader community) might play a more dominant role. Stress affected carbon cycling functions (in some soils), implying that the concept of the normal operating range holds for the detection of severe soil disturbances.

P193

Relative impacts of cow faeces and antibiotics on microbial community of grassland soils with different history of management

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Veterinary antibiotics are specifically designed to control bacteria in domestic animals. The antibiotic residues may enter the terrestrial environment when amending soils with manure. This makes them potentially hazardous to bacteria and other microorganisms in the environment. The impact of antibiotics as well as persistence of the introduced faecal microorganisms on soil microbial community is still poorly understood.

Our investigation has been focused on impact of cow faeces and chlortetracycline antibiotics (CTC) on microbial community of 3 grassland soils that differed in management history. Soils of pristine meadow (M), common pasture (P) and winter pasture (W) were sampled at the same locality Borová (South Bohemia, Czech Republic).

Mixture of faeces from 3 cows was incubated with M, P and W soils (i) either alone or (ii) in addition with a low (0.1 mg/kg) or (iii) a high (100 mg/kg) dose of CTC, in a 3-month microcosm experiment. The extended phospholipid fatty acids analysis has been used for pilot evaluation of viable biomass, structure of aerobic and anaerobic soil microbial community (SMC).

Preliminary results indicated (i) stimulation effect of faeces, (ii) inhibition effect of faeces in combination with CTC and (iii) no significant differences in CTC dose effect on soil microbial biomass. The response of SMC differed in dependence on history of soil management. While the SMC of the winter pasture soil has responded immediately, response of the meadow and pasture soils has been apparent after 14 days later.

The pyro-sequencing-based analysis of 16S rRNA genes will complete information on detail changes in diversity and structures of bacterial community.

This study was supported by Czech Science Foundation (P504/10/2077) and co-financed by Ministry of Education, Youth and Sports of the Czech Republic (-COST-CZ LD13046).

P194

Tropical soil multi-contamination: a metagenomics approach to evaluate nickel influence in petroleum biodegradation

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Question: Petrochemical industry is responsible for most of the soil multi-contamination with organic compounds and metals. The negatives effects of metals in the biodegradation of organic compounds turn the decontamination of these multi-contaminated sites challenge. Understanding the microbiological processes can help manipulate of remediation strategy. The aim of this study is to evaluate the effects of nickel on the biodegradation of crude oil in a tropical soil.

Methods: To achieve this, we did a microcosm experiment in 4 condition, soil (control), soil contaminated with oil (5% w/w), soil contaminated with oil (5% w/w) and nickel (260 mg/Kg) and soil contaminated with nickel (260 mg/Kg). These microcosms were submitted as a bioreactor conditions during 30 days. Analyses of total petroleum hydrocarbons removal were made with infrared spectrometry. Metagenomics and 16S rRNA libraries sequencing were submitted to PGM - Ion Torrent.

Results: The results showed that taxonomic changes were more affected by oil contamination (Fig. 1) and the order of Actinobacteria was predominant and increased in the presence of oil. β-proteobacteria was stimulated by oil contamination, but in the presence of metal decreased. The abundance of sequences related to organic compounds degradation increased in oil and Ni containing treatment (Fig. 2), in contrast to previous studies that shown the negative effect of metals in degradation activity *in vitro*. Even nickel caused significant changes in the microbial community, it was possible to observe a functional redundancy between β-proteobacteria and Actinobacteria (oil, oil-Ni, respectively) compared the degradation of aromatics compounds, this kind of redundancy can explain the fact that the levels of removal have not been different between treatments.

Conclusion: This soil, regardless of the changes undergone by different pollutants, was able to remove the oil accessing microbial diversity, demonstrating the importance of the preservation of genetic resources in the environment.

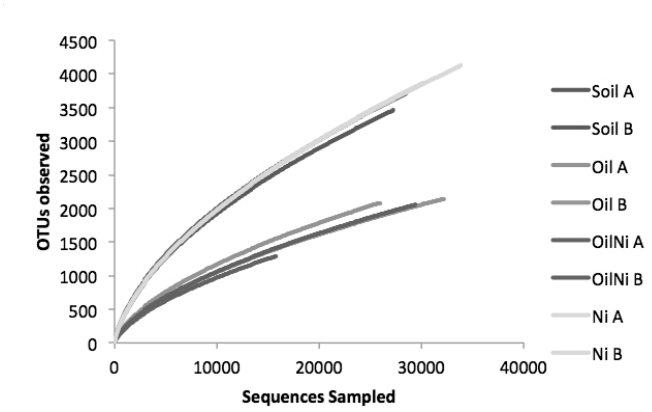

Figure 1. Rarefaction analyses of 16S rRNA libraries.

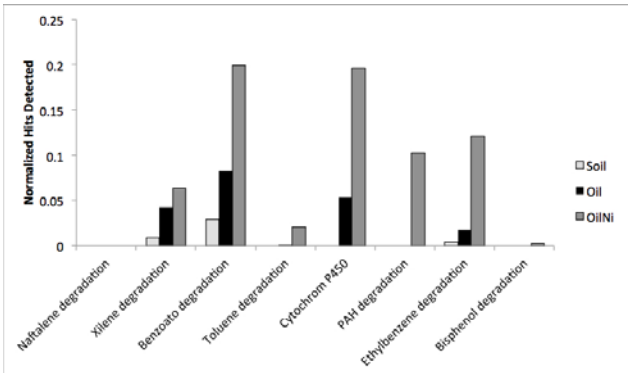

Figure 2. Relative abundance of sequences related to organic compounds degradation.

P195

tic and metabolic analysis of the carbofuran degradation pathway in *Sphingomonas* sp. KN65.2

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Introduction: Carbofuran is a carbamate insecticide that has relatively high toxicity in rats and acts as an endocrine disruptor. The compound has been banned in many countries but is still in use in developing countries. Many bacterial isolates that degrade carbofuran have been reported but only members of the genus *Sphingomonas* seem to mineralize carbofuran and use it as a carbon source. Till now, the metabolic pathway of carbofuran degradation is largely unknown and no information exists about the genes involved.

Objectives: Unravel the pathway for carbofuran degradation in *Sphingomonas* and the genes involved.

Material & Methods: *Sphingomonas* sp. KN65.2 was isolated from a vegetable field in Soc Trang province, Vietnam. Mutants affected in carbofuran degradation were obtained by screening a plasposon mutant library for growth on carbofuran. The affected genes were identified by rescue and sequencing of the plasposon flanking genes. Carbofuran metabolites were identified by high-pressure liquid chromatography coupled with linear ion trap-orbitrap mass spectrometer. The draft genome sequence of strain KN65.2 was done by Illumina sequencing.

Results: 38 plasposon mutants of strain KN65.2 were identified that showed slow and/or abolished carbofuran degradation and/or mineralization. BlastX alignment and annotation using Pfam indicated that the enzymes/proteins encoded by the affected genes function in uptake, C1 metabolism, hydroxylation and cleavage of aromatic compounds and alkanoic acid degradation. Based on the metabolite identification, a tentative pathway for carbofuran degradation was proposed, consisting of (1) hydrolysis of carbamate. (2) monooxygenation of dihydrobenzofuranol. (3) reduction of the *ortho* quinone to the corresponding catechol. (4) *meta* cleavage of the 3-substitued catechol, (5) hydrolysis of the *meta* cleavage product. (6) and (7) further alkanoc acid metabolism.

Conclusion: This study resulted into the first genetic and metabolic description of the carbofuran degradation pathway in *Sphingomonas*.

P196

Emergence and persistence of resistance genes in sewage canalization networks of metropolitan areas – unravelling the relationship between antibiotic selective pressure and antibiotic resistance genes.

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The occurrence of antibiotics in natural and urban environments seems to favour the development and spread of antibiotic resistance. At the same time the key factor for the evolution of new antibiotic resistances is the ability of an organism to adapt quickly to new environmental conditions. From an environmental health perspective, the selective pressure that antibiotic pollution may exert on bacteria is of particular concern. One of the environments which reflect the risk factors above mentioned is the water urban system with special regard to the sewage canalization and waste water treatment plants. Here, the selective pressure of pollutants and the elevated amount of microorganisms would lead to a gradual increase in the prevalence of resistance within the indigenous microbial community.

In our study raw waters originated from the sewage pipes of the main neighborhoods of Dresden metropolitan area were collected along the year 2012, the total DNA extracted and tested for the presence of different antibiotic resistance genes belonging to the chemical classes of ß-lactams, quinolones, macrolides, sulphonamides. Furthermore, the concentration of antibiotics in the raw waters was measured and the correlation between antibiotics and antibiotic resistance genes tested. In order to verify whether the water urban system under selective pressure could spread antibiotic resistances in natural ecosystems, the effluent waters of the municipal wastewater treatment plant were sampled and analyzed for their antibiotic resistance genes amount.

GIS based results revealed how the amount of resistance genes and antibiotics concentration increases in the neighborhoods with the highest antibiotics prescription rate/inhabitant.

P197

Dynamics of chlorophenol-degrading sphingomonads *in situ* by molecular and cultivation-based approaches

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Pentachlorophenol is an environmental contaminant of exclusively anthropogenic origin. Besides detoxification by methylation into pentachloroanisole, it can be mineralised through oxidative dechlorination. The only fully characterised microbial mineralisation route has been described in *Sphingobium chlorophenolicum*, and the *pcpB* gene coding the rate-limiting first step of the recently evolved degradation pathway seems to be transferred exclusively within *Sphingomonas sensu lato*. Interestingly, this chromosomal gene has been detected in sphingomonad isolates around the world regardless of the poor functionality of PcpB. Development of molecular and cultivation protocols enabling efficient detection and monitoring of sphingomonads and *pcpB* at are needed for the study of this evolutionarily exciting model system.

The aim of the presented work was to test, improve and develop nucleic acid and cultivation-based methods for the investigation of pentachlorophenol-degrading Sphingomonads and *pcpB* in chlorophenol-contaminated subsurface soils. Two sites in Finland with long-term chlorophenol contamination history were selected, and aerobic bioremediation treatment was successfully carried out at one site. Isolation of DNA and sphingomonad strains from the groundwater sediment samples were optimised. Recently suggested protocols for selective cultivation and 16S rRNA gene amplification of *Sphingomonas sensu lato* were tested on reference strains and contaminated sediments, but both failed to detect the *Novosphingobium* sp. previously identified as the prevailing pentachlorophenol degrader at the remediated site.

New *pcpB* primers with improved coverage were designed and utilised in quantification and profiling of the pentachlorophenol degrader community. Direct molecular analysis of *pcpB* seems feasible and the most efficient strategy for monitoring the dynamics of pentachlorophenol-degrading sphingomonads *in situ* and during aerobic bioremediation efforts.

P198

Evaluation of the microbial water quality and the effects of rainfall at recreational waters and sand, in Athens, Greece

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Variations in water quality at recreational bathing beaches can have significant impacts with respect to compliance with related water quality standards. The beach of Varkiza (22 kmsouth of Athens) was selected for this study due to the large number of bathers it gathers, its proximity to Athens and its 'Blue Flag' award. The microbial water quality of beaches drastically changes after rainstorms and this may have potential risks for public health. Research suggests that sand may act as a bacterial reservoir, providing a source of faecal indicators to adjacent waters; therefore, there is growing concern that public exposure to human pathogens may be underestimated.

This study is an initial evaluation of the presence of indicator microbes and pathogens at the recreational marinebeachofVarkizaand the effects of rainfall in microbial community.

Samples were collected in July 2010, February 2011 and July 2011 from knee-deep water both in dry weather and 24 h after the rainfall. Sand was collected above the high water mark. Concentrations of bacteria were calculated in terms of colony forming units (CFU) per 100 ml of water or CFU per gram of dry sand. The specific PCR primers used for 16S rDNA were F984GC and R1378 and the PCR products were separated by denaturing gradient gel electrophoresis (DGGE). The separated DNA fragments were sequenced to identify the corresponding microbial species.

Although the abundance of total bacteria seemed not to be affected by precipitation, faecal coliform and indicator bacteria concentration was significantly higher after the rainfall (*Staphylococcus* spp. 168 %, *enterococci* 71 %, *E. coli* 70 % increase) in summertime. Similar results occurred in the winter samplings (*Staphylococcus* spp. 116 %, *Enterococus* sp. 208 %, *E. coli* 14.6 % increase) although their population size was much lower. The detection of *Salmonella* was positive in all of the 6 samplings. The sand samples showed a strong presence of

Enterococcus sp. and *Candida* spp. in dry conditions only in summer. Finally, 16S rDNA DGGE sequence analysis revealed species affiliated with the genera *Novosphingobium, Ralstonia, Aestuariibacter, Glaciola, Alteromonas, Pseudoalteromonas* and *Nereida.*

P199
Influence of diclofenac on activated sludge microbial communities

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Hundreds of tons of pharmaceuticals are consumed worldwide every year. Consequently, substantial amounts of pharmaceutical residues can reach and impact on the environment, either through direct discharge or as a result of inefficient elimination in wastewater treatment plants (WWTPs). The occurrence and fate of pharmaceuticals in the water environment have attracted increasing attention due to their potential to cause undesirable ecological and human health effects. Several studies have demonstrated that some pharmaceuticals are efficiently eliminated by wastewater treatment plants (ibuprofen, naproxen, ketoprofen), while others are resistant to biodegradation (diclofenac, clofibric acid). Diclofenac (Dc) is a highly consumed non-steroidal anti-inflammatory drug (NSAID), which is often detected in wastewaters. However, investigations of its influence on and degradation by bacteria are scarce. The aim of our study was to evaluate the effect of Dc on the structure and nitrification activity of activated sludge microbial communities. The bacterial community changes in activated sludge exposed to diclofenac have been followed by terminal restriction fragment length polymorfism (T-RFLP). The influence of diclofenac on the bacterial community was detected only after 41 days of incubation with Dc and when no other sources of carbon except diclofenac were available for bacterial growth. Additionally, isolation of bacteria which are capable of growth on diclofenac as a sole carbon source was performed, and the isolates need to be further characterized for their degradation efficiencies. The activated sludge isolates were affiliated with the genera *Comamonas*, *Arthrobacter*, *Acinetobacter*, *Citrobacter*, *Aeromonas* and *Pseudomonas*, which have all been reported as aromatic degraders. Interestingly, the isolates did not represent the taxonomic groups that were identified by T-RFLP community profiling as dominant or distinctive in our diclofenac enrichments, suggesting that they might not have the dominant role in degradation of diclofenac in mixed sludge community.

P200
Change of the microbial community composition in geothermally used fluids
due to plant operation dependent temperature alterations and operation failures

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Introduction: For an efficient, reliable as well as economical use of geothermal energy the interruption of plant operation due to failures has to be prevented. Microbial metabolic activity can induce effects like mineral precipitation, mineral dissolution and corrosion, which are responsible for efficiency losses.

Objectives: To evaluate the effects of the microbial community composition and activity on plant operation dominant bacteria of a deep saline aquifer used for aquifer thermal heat storage (ATES) were identified and quantified. Special focus was put on sulfate reducing bacteria (SRB), which are known to be involved in corrosion processes. The ATES system was operated as doublet with a season- dependent fluid flow direction.

Methods: Monitoring of regular plant operation and restarts after downtime was done by using genetic fingerprinting techniques (PCR-SSCP, PCR-DGGE). To quantify metabolic groups of interest, qPCR and characterization of precipitates, like pyrite, were performed.

Results: Fluids produced from the cold well were dominated by SRB affiliated to the different genera (*Desulfohalobium* sp., *Desulfotomaculum* spp., Cand. *Desulforudis audaxviator*) and fermentative bacteria (*Anaerophaga* sp., *Halanaerobiaceae*). However, in fluids produced from the warm side sequences affiliated to Firmicutes (*Halanaerobium praevalens*), Bacteroidetes, Deferribacteres, and the Alpha-, Beta-, and Gamma- subclasses of the Proteobacteria were detected. Higher abundances of bacteria and SRB in cold fluids compared to warm fluids were proven. SRB in cold fluids probably accounted for corrosion of the well pump and iron sulfide precipitates in the nearwellbore area and topside facility filters. This corresponded to lower sulfate content in fluids. High total DNA concentrations in fluid samples taken at a plant restart decreased over time and indicated an increased abundance of microbes during downtime compared to regular operation.

Conclusion: A temperature decrease of 40 K led to a higher abundance and diversity of SRB in the aquifer fluids, thus microbially mediated processes adversely affected plant reliability. Results are published in Lerm et al. (2013), Thermal effects on microbial composition and microbiologically induced corrosion and mineral precipitation affecting operation of a geothermal plant in a deep saline aquifer. Extremophiles.17(2):311-27.

P201
Directed evolution of radiation-resistant acidophiles

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Introduction: Numerous Cold War-era radioactive storage tanks in the United States have leaked into the environment. The mixed wastes are highly radioactive and acidic. Bioremediation with radiation-resistant acidophiles represents a cost-effective strategy for cleanup of contaminated sites. Acidophiles are organisms that have pH optima for growth at or below pH 3.0 and are most widely distributed in the bacterial and archaeal domains. Mechanisms, by which acidophiles deal with low pH, include

impermeability of cell envelope to protons, reversed membrane potential, and intracellular buffering.

Objectives: There have been no reports of acid-tolerant, extremely radiation-resistant organisms, and the goal of this research is to i)apply directed evolution to selecting acidophilic *Deinococcus* bacteria, which are extremely radiation-resistant; ii)isolate naturally acidotolerant and radiation-resistant microorganisms from the environment; and iii)screen acidophiles from different collections for radiation resistance.

Materials and Methods: One approach (acute) to directed evolution of *Deinococcus* for acid-tolerance applies successive rounds of exposure to pH 2, followed by recovery of survivors. A second approach (chronic) selects for deinococcal acid-resistance under increasingly acidic conditions.

Results: To date, we have selected strains of *D. radiodurans* and *D. geothermalis* that can survive exposure to pH 2 for 4 min and 12 min, respectively. By comparison, the founder strains survived 40 s and 1 min. Chemical mutagenesis with N′-methyl-N′-nitro-N′-nitrosoguanidine and 5′-azacytidine was applied, but has not yet yielded acidotolerant *Deinococcus* mutants. From almost 80 samples of desert soil, and acid mine drainage water and soil samples, we isolated 30 acidotolerant/acidophilic strains. Two of strains were also extremely radiation-resistant, capable of growth under 124 Gy/hour at pH 2.5. DNA analysis identified both strains as basidiomycetous yeasts that belong to the genus *Rhodotorula*. From 10 screened acidophilic bacterial species only *Acidithiobacillus ferrooxidans* showed radiation resistance.

Conclusion: Radiation-resistant acidophiles are now being tested for transformability and for their ability to express heterologous cloned genes under high radiation and low pH.

P202
Seasonal changes in bacterial and viral population in a waste water plant efluent

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[4]National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia
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[6]Wastewater treatment plant, Domžale-Kamnik, Domžale, Slovenia

Changes of bacterial and viral populations were monitored monthly for the duration of one year in the effluent of a single waste water treatment plant (WWTP). The composition of entire bacterial population was determined by denaturing high performance liquid chromatography (DHPLC) of amplified 16S rDNA gene. Additionally, bacteria from genus *Clostridium* were cultivated and strains identified as *C. difficile* were further typed into ribotypes. Viral populations were analysed after concentration of 5 L volumes with CIM-QA 8ml tube columns (BIA separations), followed by real time quantitative RT-qPCR detection of rotaviruses, astroviruses, noroviruses GG I and II, sapoviruses and hepatitis A virus.

By DHPLC 35 different bacterial genera/species were identified and the overall bacterial population patterns seem to be stable for few months but changing through the year. Eighteen different clostridal specias were isolated, but only *C. difficile*, *C. perfringens, C. butyricum* and *C. thiosulfatireducens* were present in three or more samples. Overall, 17 different *C. difficile* ribotypes were present in WWTP effluent, but only the ribotype 014/020 was detected in all samples. This ribotype is among three most prevalent types in Slovenian human patient isolates.

All monitored enteric viral pathogens were present throughout the whole year, except for hepatitis A virus, which was never detected. Rotaviruses are the viral pathogen that is released at higher concentrations in the effluent, followed by Sapovirus, Norovirus GG-I, astrovirus and Norovirus GG-II. For some of the viruses, especially for Norovirus GG-II, the concentration step resulted essential for its detection. Astroviruses were not detected in August and September even after concentration step. In contrast, in those two months rotaviruses were detected in highest concentrations. This could be associated with the water temperature, or with a higher/ lower prevalence during those months.

Our results show that the overall bacterial population in WWTP effluent varies in time, but the bacterial and viral intestinal pathogens are present throughout the year.

P203
Micro fluid segment technique – application in microtoxicology and potential for investigation of interaction of microorganisms

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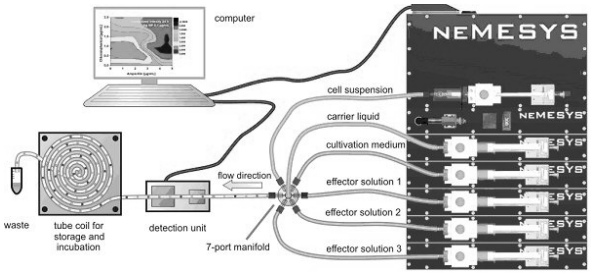
Micro fluid segment technique has been proved as a powerful instrument for generation of large numbers of separated cell cultures. These sequences of micro fluid segments can be used for determination of highly resolved dose response functions as well as for the investigation of combinatorial effects. The technique was applied for investigation of growth of chlorella in the presence of different toxins. So, it can be shown that synergistic effects take place in combinations of atrazine with silver nanoparticles.

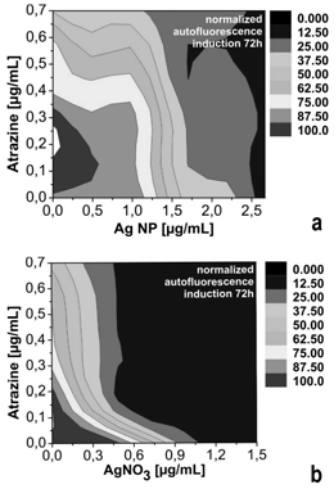
The bolographic maps are well suited for the characterization of a dose-dependent complex response behaviour. This is particular important in case of antagonistic and stimulation effects. So it could be shown that antibiotic activity of ampicilline was significant effected by combination of caffeine and chloramphenicol.

Despite the micro toxicological investigation, the micro segmented-flow technique is a very promising method for investigation of cellular interaction. On the one side, the small test volume is interesting for studying effects with low amounts of substances released from a cell without getting in high dilution. On the other site, it allows the systematic variation of one, two or three parameters effecting inter-organismic relations. So, experiments with step-wise variation of ratios of starting cell numbers, substrate concentrations and effectors can be executed

in order to screen complete multidimensional parameter fields under conditions of a restricted, but well-defined „microecological situation".



Screening principle/experimental realization



Examples for different response behaviour of cells on combinations of drugs: a) independent activity: silver nanoparticles (silver ion-equivalent concentration) and atrazine b) synergistic behaviour: silver ions and atrazine

P204

A *Rhizobium tropici* SEMIA 4077 mutant efficiently produces an exopolysaccharide of bioremediation interest

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Introduction: Bioremediation, a strategy mediated by microorganisms, is a promising method to degrade or remove organic contaminants from aquatic systems or soil. Exopolysaccharides (EPS), which are produced by a variety of Gram-negative bacteria, have been shown to be potential bio-emulsifiers for use in the degradation of hydrocarbons.

Objectives: The goal of the present study was to inactivate some components of the EPS gene cluster from the bacterium *Rhizobium tropici* SEMIA 4077 using transposon insertional mutagenesis. Attempts were made to optimize the production of EPS using mutant bacterial strains.

Material and Methods: The quantities of EPS produced by the wild-type and mutant strains of *Rhizobium tropici* SEMIA 4077 were determined from shake-flasks cultures grown under different nitrogen and carbon levels. After incubation, the supernatants of the individual bacterial strains were collected, and the EPS was extracted by alcohol precipitation. To determine the monosaccharide composition of the EPS, crude EPS samples were analyzed by HPLC using 1-phenyl-2-methyl-5-pyrazolone.

Results: The *R. tropici* 4077::Z04 mutant produced the largest quantity of EPS when compared to the wild-type and other mutant strains, which it achieved through greater efficiency. The ratio between the total EPS production and the cellular biomass was 84.05 for the 4077::Z04 mutant and only 8.085 for the wild-type strain. A chromatographic analysis revealed the presence of a heteropolymer with neutral to acidic sugars in the EPS composition.

Conclusions: This study determined that the mutants obtained through insertional mutagenesis had greater EPS production than the wild-type strain. Efficient production is an advantage because these products act as depollution agents, and there is a growing interest in compounds that perform bioremediation.

Keywords: exopolysaccharide, *Rhizobium*, mutants, bioremediation

P205

Topoisomerase IV is required for circular chromosomes but not linear chromosomes in *Streptomyces*

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The soil bacteria *Streptomyces* possess both linear chromosomes and linear plasmids. The linear chromosomes are relatively unstable, undergoing frequent circularization spontaneously. Theoretically, the circular chromosomes need a decatenase for post-replicational segregation of the daughter molecules, but the linear replicons may not. *Streptomyces* chromosomes contain two genes, *parC* and *parE*, that encode the subunits for the decatenase Topoisomerase IV (Topo IV). Moreover, the telomeres of the linear chromosomes and plasmids of *Streptomyces* interact *in vivo*, resulting in the formation of superhelical circular configuration of these linear replicons. If such circular configuration persists through replication, it may require Topo IV for decatenation. On the other hand, eukaryotic chromosomes, despite their linear topology, require a type-II topoisomerase, Topo II, for post-replicational untangling of the daughter chromosomes. We set out to address the question whether Topo IV is required for separation of the linear chromosomes and linear plasmids in *Streptomyces*. Initial attempts to delete *parE* on the chromosome of *Streptomyces coelicolor* were not successful until a functional *parE* was provided on a temperature-sensitive plasmid. Subsequently, the plasmid was eliminated at high temperature, and *parE* deletion mutants were obtained. These results indicated that Topo IV was not required for viability. Presumably the telomere-telomere association can be resolved during or after replication to achieve decatenation. Nevertheless, the *parE* mutants exhibited retarded growth, defective sporulation, and temperature sensitivity, which may reflect inefficient decatenation through the dissociation of the telomere-telomere complexes on the linear

chromosomes. Moreover, in the *parE* mutants, circular plasmids could not replicate, and the spontaneous circularization of the chromosome could not be observed. Our results, thus, show that Topo IV is absolutely required for decatenation of the circular replicons in *Streptomyces*, and also provides a more efficient mechanism for post-replicational untangling of the linear replicons.

P206

Functional screening of a winter and a spring genomic DNA libraries obtained from soils in a winter wheat crop

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Soils are very rich environments where the diversity of microorganisms is very high. These microorganisms play important role in the degradation of organic matter with enzymes able to degrade it. The aim of this work is to discover by functional screening new enzymatic activities of microorganisms from soils collected in winter and spring in a winter wheat crop. The genomic DNA was extracted from both soils to construct two libraries in *Escherichia coli*. These libraries were then screened for several enzymes such as lipase, beta-glucosidase, cellulase, α-amylase,... At this time, 2 beta-glucosidases and 3 lipases have already been found in the winter library and 3 beta-glucosidases and 1 lipase in the spring library. Sequence analyses with the BLASTX program revealed that two beta-glucosidases have less than 65% of sequence identity with known beta-glucosidases, one have 64% of identity with a known beta-galactosidase and one have 59% of identity with a glycoside hydrolase. The fifth seems to be a phosphorylase kinase (54% identity) which have a glucoamylase domain responsible for the activity. This ORF is interrupted by a transposase. Three of the four lipases have less than 60% of sequence identity with known lipases/esterases. The fourth show 55% of identity with a known beta-lactamase.

P207

Correct OTUs - survey of different denoising and OTU clustering tools for amplicon sequence data

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Next generation sequencing techniques have become an essential tool in studying diverse microbial communities. Typically one to three variable regions of 16S rRNA gene are amplified and the fragments are sequenced. Massive amounts of data are generated, and analysing these data has become the next challenge. There are a variety of tools available for data analysis and several research groups are developing new methods. However, using different algorithms gives differing results, particularly in numbers of OTUs and diversity estimates.

Here we compare different denoising and clustering algorithms using published datasets with well-known community structures. We applied Denoiser, Mothur shhh.flows and Acacia for denoising, and the reads were clustered to OTUs using Uclust and CD-HIT in Qiime, Mothur average neighbour, ESPRITTree and BEBaC. Taxonomic affiliations were defined using BLAST algorithm against Greengenes taxonomy. The aim was to grasp which algorithms and parameters work most reliably and compare the results to non-sequencing based community characterization.

The results show drastic differences between OTU numbers derived from different data analysis methods. Both denoising and clustering algorithms affected the number of observed OTUs: denoising algorithms by twofold and clustering methods by more than one order of magnitude. We also observed the selected minimum read length impacting the number of OTUs substantially when sequence data was not denoised but trimmed based on quality scores.

The taxonomic affiliations were also affected by the choice of data analysis methods. The most abundant OTUs were detected by all methods but in different relative abundances. Differences were found in the presence of rare taxa which varied between denoising and clustering methods. The biggest differences were seen even at phylum level.

Our results support the observation that different analysis methods give dissimilar results, even at high taxonomic levels. It's fundamentally important to meticulously describe in publications how the sequence analysis was performed because the results can be highly dependent on the methods and parameters. The differences between data analysis methods should be acknowledged when comparing the results to other studies.

P208

Systems biology approaches predict inorganic N regulation of *xylM* and *xylE* gene expression and xylene degradation by *Pseudomonas putida* mt-2 in soil microcosms

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Prediction of effective biodegradation of BTEX compounds in oil-contaminated soil is hampered by inadequate understanding of catabolic gene regulation as affected by environmental stimuli. Systems biology approaches have revealed complex regulatory circuits, in which environmental stimuli such as nutrient availability modify/modulate the induction of bacterial catabolic genes by pollutants. This study evaluates the effect of inorganic N-sources on the induction of *xylM* and *xylE* genes in *Pseudomonas putida* mt-2.

Induction of *xylM* and *xylE* genes, monitored by quantitative RT-PCR at high temporal resolution, was linked to ^{14}C *m*-xylene mineralization in both pure cultures using NH_4^+ as the N source, and in soil microcosms amended with 10^7 cells g $soil^{-1}$. This demonstrates that *xylM* and *xylE* expressions are robust descriptors of xylene degradation *in situ*. Shifting the N source from NH_4^+ to NO_3^- resulted in delayed but subsequently stimulated *xyl* gene expressions in pure culture. Amendments with NO_3^- (rather than NH_4^+) in N-limited soil microcosms likewise stimulated both *xyl* gene expressions and m-xylene mineralization in soil. N-limitation in the soil was verified by biosensors responding to N starvation. In addition, comparable cellular responses to N-availability (NH_4^+versus NO_3^-) were demonstrated for cells in pure cultures and soil microcosms by expression analysis of the genes *amtB* and *gdhA*.

These results provide the first indications that systems biology approaches predicting catabolic gene expression can be applied to a simple soil model system. Further, the model system should be useful for testing the impact of additional environmental factors on *xyl* gene expression.

P209
Understanding the catalytic mechanism for (p)ppGpp synthesis by Rel proteins

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Introduction: A hyper-phosphorylated guanosine molecule, (p)ppGpp, synthesised by Rel proteins, is a master regulator for stringent response mechanism. Rel proteins include monofunctional RelA enzymes that are (p)ppGpp synthetases and bifunctional SpoT/RSH (Rel Spo Homolog) proteins, possessing both (p)ppGpp synthesis and hydrolysis activity.

Objective: Our focus has been to understand the catalytic mechanism of Rel proteins in (p)ppGpp metabolism.

Materials and Methods: Analysing the sequences of Rel proteins, we found different motifs in the synthetase domain of monofunctional and bifunctional enzymes. Biochemical analysis was carried out to probe functional importance of these motifs.

Docking ATP into the active site of modelled N-terminal Rel protein structure, we identify a putative ATP binding site. Highly conserved amino acid residues near the identified ATP binding site were mutated in the N-terminal fragment of Rel *Mycobacterium tuberculosis*, and their effect on (p)ppGpp synthesis was studied.

Results: Contrary to earlier reports on bifunctional Rel proteins, for monofunctional RelA from *Escherichia coli* we did not find inhibition of the (p)ppGpp synthesis at higher Mg^{2+} concentrations. We attributed this difference synthesis activity to the charge reversal in the synthetase domain. The bifunctional Rel proteins have RxKD motif, whereas ExDD motif is present in *E. coli* RelA which is a monofunctional enzyme. We argued that later uses two metal ion mechanism for (p)ppGpp synthesis. We showed that these motifs are also important in determining substrate specificity (GTP/GDP), cooperativity and regulation of catalytic activities of the N- terminal region via the C-terminal region. Besides this, we found that Rel proteins carrying an EXDD motif synthesize a novel metabolite, pGpp; whose biological importance is not yet understood.

Out of the conserved residues in the synthetase domain we find that a subset of these residues co-ordinate the catalytic metal ion Mg^{2+}, and another subset stabilise the transition state of the (p)ppGpp synthesis reaction.

Conclusion: Overall, we suggest a likely catalytic mechanism by which Rel proteins synthesize stringent response molecule i. e. (p)ppGpp.

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P210
Analysis of metagenomic sequences from brazilian mangrove sediments with potential for enzymatic activities

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Mangroves are considered one of the richest ecosystems in the world due to its intense biological activity. These environments constitute, in general, an extreme habitat for microorganisms due to the high salinity, lack of oxygen, deficiency of nutrients, pH variations, among other adverse conditions. Microbial communities in mangroves are supposed to be adapted to these rough conditions, and may constitute a source of active molecules with particular properties of great interest for industrial processes. However, researches involving microbial diversity in these environments are scarce and, in this sense, the purpose of this work was to explore the potential of the microorganisms from mangrove sediments using metagenomic approach and high throughput screening tools to identify enzymatic activity.

A metagenomic library was constructed using the "Cloning-Ready Copy Control pCC2FOS" kit (Epicentre®) and a total of 672 clones were subjected to high throughput screening for peptidase activity based on the use of Abz probe, as described by Oliveira *et al.* (2012). Fifteen positive hits were obtained and the clone with the best result was selected for full insert sequencing using the Ion Torrent™ platform. Putative enzymes were identified by comparison of deduced aminoacid sequences with protein databases (RAST, BLAST), and included CTP synthase (65% identity), alanine dehydrogenase (63% identity), among others. According to literature, these enzymes are involved in catalytic reactions related to different functions such as pyrimidine biosynthesis and deamination of L-alanine to pyruvate. These results may offer a more comprehensive view of the poorly known ability of the microbiota from mangrove systems as a source of novel biocatalysts with diverse activity of interest in biotechnological processes. Further steps of the work will comprise clone insert digestion and ligation into an expression vector for activity analysis and further chemical characterization.

P211
Characterization of new bacterial glycoside hydrolases isolated from agricultural soils using a functional metagenomic approach

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Microorganisms play key roles in soil ecosystem functioning, notably through their ability to degrade plant cell wall polymers. For this, bacteria and fungi produce various enzymes such as cellulases, xylanases, glucosidases, esterases or laccases. Finding new enzymes hydrolyzing cellulose, hemicellulose or lignin is not only interesting for a better understanding of the roles of the soil microflora still largely unknown but these enzymes are also

useful for various biotechnological applications such as the production of renewable energy from lignocellulosic material. So here, we used a functional metagenomic approach to isolate new bacterial β-glucosidases, which were then biochemically characterized. The new enzymes were identified by functional analysis of agricultural-soil metagenomic libraries hosted in *Escherichia coli* and screened on medium containing esculin. After sequence analysis and preliminary estimation of the activity of the new β-glucosidases using p-nitrophenol derivatives on intact bacterial cells, the coding sequences of three of them were cloned into a bacterial expression vector so as to overproduce and purify them by affinity chromatography. The chosen enzymes show only 52-64% sequence identity to known family 3 (GH3) or 1 (GH1) glycoside hydrolases of different phyla (Actinobacteria, Acidobacteria and Proteobacteria). Analysis of the *E. coli* cells expressing each of them revealed that both GH1 proteins (ASEsc9 and ASEsc10) are thermophilic enzymes more active at mildly acidic to neutral pH while the GH3 enzyme (ASEsc6) is an alkaline, mesophilic, β-glucosidase also displaying xylosidase activity. Their coding sequences have been cloned in fusion with a carboxy-terminal His-tag and placed under the control of the IPTG-inducible promoter of the pET-30b vector. The proteins will be overproduced and purified for further characterization.

P212
DNA sorption blocker "G2" increase DNA recovery from subsoil clay sediment >1.000 times

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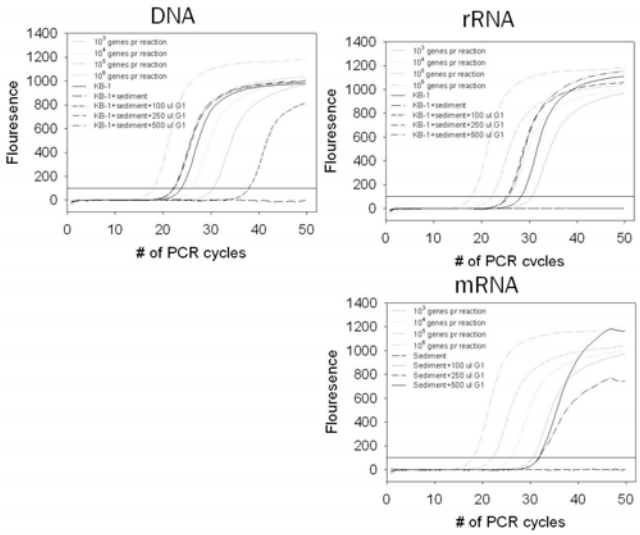
One of the obstacles when doing molecular microbial ecology studies in low biomass clayey sediments is a very low recovery of nucleic acids. The aim of the present study was to develop a suitable extraction procedure for DNA and mRNA from clayey sediments, and to apply this in order to investigate microbial dynamics based on mRNA, as well as DNA.

The sorption blocking reagent G2 was developed from heavy modifications of nucleic acids. This reagent was added to soil samples while extracting DNA, for the purpose of saturating sorptive sites in a clay soil before lysis of cells.

With the development of the G2 blocking reagent, we were able to optimize a suitable nucleic acid extraction protocol for these difficult sediments with a >thousand-fold increase in extraction yields. Using this extraction protocol, we obtained high resolution expression profiles of the functional genes *vcrA*, *bvcA*, and *tceA*. A ring test of G2 was conducted, in which 12 different laboratories extracted DNA (with and without G2) from a sample soil and returned the DNA to our lab for qPCR quantification of the *rpoB* gene. The multi-laboratory test confirmed that adding G2 sorption blocker, when extracting DNA from low biomass clayey sediments, increases the yield of the extraction significantly.

The blocking reagent is being commercially developed and is currently available at the cost price from GEUS formulated in 2 ml beadbeating tubes with either 0.1 mm glassbeads or 1.4 ml ceramic beads as lysing matrix.



P213
Effects of static magnetic field on model wastewater bacteria and on ammonium removal from wastewater

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Biological reactions in living systems under low static magnetic fields (SMF) have been studied by many researchers, but the effects of magnetic field (MF) on microorganisms are still poorly understood and studies addressing this question are scarce. The observed effects published in the literature appear to be dependent on various factors such as the strength of the MF, the bacterial strain, the composition of the growth medium and the growth temperature. We recently showed that SMF (B=17 mT) inhibits the growth of *E. coli and P. putida* and increases dehydrogenase activity and also ATP levels in exposed bacteria. The effect was transient and lasted only as long as the cells were exposed to SMF (Filipič et al, 2012). Recent reports suggested that SMF may also stimulate removal of organic substrates from wastewater, which lead us to examine whether ammonium removal by sludge bacteria added to the municipal wastewater or by *Nitrosomonas europaea* grown in the laboratory culture could be stimulated by static magnetic fields (SMF). Results showed for the first time that SMFs of 30 and 50 mT (but not 10 mT) increased wastewater treatment processes in sequencing batch bioreactors (SBRs) by dramatically increasing the ammonium oxidation rate by up to 85%. In addition, ammonia oxidation activity of *N. europaea* increased when exposed to 17 mT SMF. SMF stimulated growth of ammonium oxidizers within a mixed wastewater microbial community, which increased by 40%, and of *N. europaea*, which increased by 60% after 7 days of the SMF treatment respectively ,in comparison to the non-exposed cultures. Results of this work therefore suggest that SMF could be applied to increase ammonium removal rates or stimulate the growth of ammonia oxidisers in biological wastewater treatment plants.

Filipič et al., Bioresource Technology 120 (2012), 225-232

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P214
Pan genome analysis of *Lactobacillus crispatus*

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Lactobacillus crispatus is a ubiquitous micro-organism that can be recovered from a range of host-associated habitats. It is commonly associated with the beneficial gastrointestinal microbiota of animals and it is also one the most prevalent species of the *Lactobacillus*-dominated human vaginal flora. Moreover, *L. crispatus* contributes to the urogenital health of the host by producing antimicrobial agents and through competitive exclusion. In order to investigate the genetic diversity of this central urogenital species, we performed a comparative analysis of the currently available *L. crispatus* genome sequences.

Exploiting the completed genome sequence of strain ST1 (1) and the draft genome sequences of nine other *L. crispatus* isolates (2), we defined the scale and scope of the pan and core genomic potential of *L. crispatus*. Using OrthoMCL (3), the full complement of *L. crispatus* protein-coding genes were assigned to 3929 orthologous groups, nearly twice the size of an individual genome. Approximately 30% of these orthologous groups were universally conserved among the isolates, forming the common core. Mathematical modelling indicated that the strains have an open pan-genome. New gene families will be discovered and the core-genome continues to decrease as more isolates have their genomes defined. Moreover, whole-genome comparisons depicted high sequence identity and extensive synteny, and the alignments revealed that on average approximately 90% of each strain's genome is conserved.

This pan genome analysis provides new insights into the intraspecific genome variability and the collective molecular mechanisms of the species *L. crispatus*. Using this approach, we described the differences and similarities between the genomes and identified strain-specific features. Notably, on average close to 60% of the orthologous groups of a given strain belonged to the current core, whereas around 6% were strain-specific. Largely, these strain-specific groups were involved in, for example, transportation, regulation of transcription, and phage-resistance.

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P216
Abundance of *mcr*A and CH_4 emissions from different vinasse distribution systems in a Brazilian sugarcane mill

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Ethanol from sugarcane has a high favorable energy balance and has a great production and commercial potential for fast expansion in many countries. In the ethanol generation process there are produced some co-products. Vinasse is one of these co-products, and can be used as soil fertilizer. However, there are few studies about possible environmental impacts due to the vinasse application regarding to greenhouse gas (GHG) emissions. It is already known that the greatest impact from the vinasse application is the emission of CH_4. Meanwhile, studies concerning the methanogenic microorganisms involved in CH_4 emission are still needed. Thus, the main goal of this study was to measure the CH_4 emission and quantify the abundance of *mcr*A gene (enzyme complex present only in methanogenic *Archaea*) from different vinasse distribution systems. CH_4 and sediment samples were taken in different seasons (beginning, middle and end of sugarcane harvest) at two different distribution systems - uncoating open channel (UC) and coating open channel (CC). The abundance of *mcr*A genes were assessed by qPCR and correlated to CH_4 emissions, which was measured by gas chromatography. It was observed significant difference in CH_4 emission between UC and CC samples in all seasons, where the higher emission it was reported in UC samples. Similar trend occurred to *mcr*A gene abundance, which was higher in UC samples. The lower emission and gene abudance observed in CC samples was associated to high vinasse temperatures (nearly 50°C), which showed to be more detrimental to methanogenesis, other than channels characteristics, such as the channel coating. The higher emission of CH_4 in UC in all seasons can be associated to anaerobic conditions and high content of organic matter. In UC samples the vinasse temperatures varied between 24 and 30°C, which is the optimum temperature for methanogenesis. Higher emissions of CH_4 were observed in UC in the middle of the sampling period but higher *mcr*A abundances occurred in UC at the end of the harvest. It was observed a significant correlation (Tukey, $p>0.05$) between CH_4 fluxes and abundances of *mcr*A genes. The results showed significant differences ($p>0.05$) in the abundance of *mcr*A, suggesting this archaeal gene can be used as indicator of methanogenesis potential and as well as indicator of biological CH_4 emission.

P217
Comparative genomics of the meat spoilage bacteria *Leuconostoc gelidum* and *Leuconostoc gasicomitatum*

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Introduction: *Leuconostoc gelidum* and *Leuconostoc gasicomitatum* are common meat spoilage bacteria that often dominate in late shelf-life, modified atmosphere packaged meat. Whole genomes of one *L. gelidum* and one *L. gasicomitatum* strains have been published so far, but the full genomic diversity of these species remains unsolved.

Objectives: Comparative genomic analyses within the species *L. gelidum* and *L. gasicomitatum* was performed in order to better understand genome evolution and meat spoilage potential of these bacteria.

Materials and methods: Four bacterial isolates from meat and four isolates from vegetable sources, all representing species *L. gelidum* or *L. gasicomitatum*, were chosen for next generation sequencing with Illumina HiSeq2000 Platform to generate draft genomes. The

genomes were assembled using Velvet and annotated using RAST. OrthoMCL algorithm was used to group orthologous protein sequences.

Results: Comparative genomic analyses of the eight new draft genomes of *L. gelidum* and *L. gasicomitatum,* and the publicly available whole genomes of *L. gasicomitum* LMG 18811T and *L. gelidum* JB6 was performed. Considerable variation in the number of predicted open reading frames and in the coding potential of the strains was discovered. The genetic basis of the ability of the *L. gelidum and L. gasicomitatum* strains to grow in meat/vegetable environment was investigated.

Conclusion: The core and pan genome of the species *L. gelidum/gasicomitatum* was defined, and the genomic potential and adaptation of these bacteria to meat environment was elucidated.

P218
Quantification of antibiotic resistance gene input and output in wastewater treatment plant

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Antibiotic resistance is increasing rapidly among bacteria as antibiotics are used in increasing quantities in treating humans and animals. Wastewater treatment plants are known reservoirs for various resistance determinants and serve as hotspots for horizontal gene transfer. The resistant genes from the wastewater treatment plants may eventually end up in the environment either in the effluents or in the sludge. One major concern is the microbial resistance against last resort antibiotics, such as vancomycin, cephalosporins, carbapenems and quinolones.

Though antibiotic resistance has been widely studied at wastewater treatment plants, there are few studies characterizing more than few genes or studying the fate of the resistance genes after the purification process.

Our aim was to characterize and quantify the antibiotic resistome in the wastewater treatment plant and to estimate the release of resistance genes from the process to the environment. The resistance genes were quantified with real-time PCR from influent and effluent waters, sludge collected from the process and sediments near the release site of the effluent waters.

The results show that many resistance determinants can be found in the wastewater influents and that the resistance genes are also found in the effluents and dried sludge from which they are released to the environment.

P219
Allies and foes – community ecology of bacteria and their bacteriophages in plant diseases of orchids

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Introduction: Bacteriophages garnered considerable interest in the past years as a potential way for biocontrol of plant pathogenic bacteria for which no efficient chemical control exists. While efficient under experimental conditions with defined starting concentrations of both bacteria and their phages, little is known about phage dosing and the various parameters influencing the outcome of bacterial-phage interections in nature. The objective of our study is to develop tools for studying the interections among selected pathogenic and non-pathogenic bacteria and their bacteriophages in orchid plants.

Materials & Methods: Pathogenic and non-pathogenic bacteria were isolated from diseased (rotted) *Phallenopsis* orchids from commercial production. Pathogenic bacteria were identified by classical microbiological methods, real-time PCR assays and sequencing of fliC gene. Specific bacteriophages active aginst these bacteria were isolated through enrichment on target bacteria.

Results: Pathogenic bacteria isolated from rotted orchid tissues were identified as *Dickeya* spp. however, they were shown to differ from currently described species of *Dickeya* in nucleotide sequence of fliC and represent UDL-3 and UDL-4 lineages of these bacteria as described by Van Vaerenbergh *et al.* (2012). These differences in fliC gene enabled us to develop strain specific real-time PCR assays and quantify these bacteria in plant tissues. Specific bacteriophages against them and non-pathogenic bacteria present in orchid tissues were isolated and tentatively classified as Myoviridae and Podoviridae based on transsmission electron microscopy. Inhibition experiments showed that pathogenic bacteria are able to inhibit non-pathogenic bacteria through excretion of inhibitory substances. Determination of bacteria and bacteriophages in diseased and healthy plants is in progress.

Conclusion: Little is know about community dynmaics of pathogenic and non-pathogenic bacteria of orchids and their phages in nature. We describe the development of tools enabling such studies of a chosen model system. Studies are in progress of bacterial concentrations and phage titers at various time points in in vitro co-cultivation, after artificial orchid inoculation experiments and in naturally infected orchids.

P220
Implications of interaction between human pathogen *Campylobacter jejuni* and its bacteriophages

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Bacteriophages are natural predators of bacteria and can be found in almost every ecosystem where their hosts are present. Their are very specific, in most cases their host range is limited to strains of one bacterial species. *Campylobacter jejuni* is most common reported cause of zoonosis in EU (EFSA, 2010). Poultry is considered to be the main source of infection, because *C. jejuni* colonizes their intestine to high level. It has been proven that a 100-fold decrease of *C. jejuni* contamination would result in 30-fold reduction in the incidence of *C. jejuni* infection. Bacteriophages have been proven to reduce number of *C. jejuni* in broilers before slaughter for 1 - 4 $\log_{10}$. However, for successful implementation of bacteriophages as antimicrobial agent,

a deeper understanding of *Campylobacter* bacteriophage biology and ecology is needed. A set of *Campylobacter* specific bacteriophages has been isolated from poultry and pig samples. They all show similar morphology and belong to *Myoviridae* family. They could be distinguished by their genome size, DNA restriction profiles and their ability to infect *C. jejuni* strains of human, poultry, pig and environmental origin. Molecular characterisation of interaction between *Campylobacter* bacteriophages and their hosts has shown that capsule is required to initiate infection of host bacteria. Active flagella are also important in infection process, but this interaction is independent of type of flagellar glycosylation present. Capsule and flagella are phase variable structures and vary within a strain, which is reflected also in rapid bacteriophage resistance development in *in vitro* experiments. *In vivo* trials have shown that resistance arises also due to rearrangement of prophage sequences in genomic DNA of *C. jejuni* (Scott et al., 2007). *C. jejuni* strains have been found to carry prophage related sequences in their genomes, especially human isolates, however, prophages could not been isolated from these strains by mitomycin C induction.

P221
Polyphasic taxonomy of actinomycetes isolated from marine environment – Multilocus Sequence Analysis (MLSA) x DNA-DNA hybridization

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Members of the Actinobacteria class have considerable value as prolific producers of biologically active secondary metabolites and marine actinomycetes have yielded numerous novel secondary metabolites and new actinomycete taxa of marine origin have also been recovered[1]. *Multilocus Sequence Analysis* (MLSA) has been considered an important and more accessible tool for assessing phylogeny and taxonomy of prokaryotes, increasing the inference obtained by phylogenetic analysis of 16S ribosomal RNA gene[2]. Studies have shown that the comparison of multiple genes sequences that encode proteins is useful for the delineation of species and could take the place of DNA-DNA hybridization (DDH) required to define a new species[3]. The purpose of this work was to screen the genetic diversity of 71 actinomycetes, isolated from marine macroorganism, collected from north coast of São Paulo State, Brazil, based on the analysis of 16S rRNA genes and evaluation the potential of MLSA (*rec*A, *rpo*A, *rpo*B, *trp*B and *gyr*B genes) for taxonomy and classification of strains and comparison with DDH data. Genes were amplified by PCR and phylogenetic trees based on the single and concatenated 16S rRNA and housekeeping gene sequences were constructed and DDH was performed according to Menezes et al[4]. Each single-gene tree had an overall topology similar to that of the concatenated sequence tree. All genes used in this study proved to be good molecular markers, were well consistent with the 16S rRNA gene analysis. High correlation between MLSA and DDH was observed, in both single and concatenated sequence phylogeny, showing good relation between the techniques.
Acknowledgements**:** Fapesp

P222
Ammonia concentration as the major driver of niche specialisation between AOA and AOB

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Nitrification plays a crucial role in global N cycling. Ammonia oxidation, the first and rate-limiting stage of nitrification, has been demonstrated to involve both ammonia-oxidizing bacteria (AOB) and archaea (AOA). The AOA:AOB ratios in environmental samples vary strongly from one study to another, ranging from sensu strictoto niche partitioning. Recent evidence strongly suggests AOA niche specialisation for oligotrophic environment, i.e. when ammonia concentration is low. Indeed, cultivated AOA exhibit very high affinity for ammonia, with K_m from 1 to 4 orders of magnitude lower than those of AOB. This is consistent with the increase of AOA:AOB ratio as soil pH decreases, i.e. when the equilibrium between ammonium and ammonia shifts towards ammonium. Niche differentiation between AOA and AOB could even extend to their nutritional strategies, with several indications that AOA may exhibit heterotrophic or more probably mixotrophic growth. More particularly, urea degradation might be a pathway to meet both AOA carbon and energy demands. This study was based on the hypotheses that (i) ammonia concentration is the major factor driving the relative abundance and activity of AOA and AOB in soil and (ii) AOA are favoured in soil where urea is the only available source of ammonia. To test these hypotheses, soil slurries were established with five different ammonium or urea concentrations, ranging from 1 to 10,000 µg N ml^{-1}. The differential influence of ammonia and urea concentrations on AOA and AOB was determined by short-term (15 min to 12 h) expression of AOA and AOB *amoA* genes, encoding ammonia monooxygenase, and *ureC* genes, encoding the urease α subunit and longer-term (1 to 7 day) growth, monitored as increases in *amoA* gene abundance, and changes in ammonium, urea and nitrite concentrations.

P223
Dermocosmetics – microbial diversity in raw materials and finished product

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The presence of microorganisms in pharmaceuticals products, not only threats consumers' health but affects negatively the product's characteristics. The aim of this study was the investigation of microbial diversity in raw materials and finished product of a pharmaceutical industry, using a combination of culture dependent techniques and molecular methods.

The study was focused towards the purified water, which constitutes the basic ingredient in the formula of the sunscreen and it was focalized in the detection and isolation of heterotrophic and oligotrophic mesophilic community, psychrophilic community and filterable bacteria that penetrate the filter membranes with 0.2 µm diameter pores.

The results of this study demonstrate two discrete communities which prevailed in purified water. The heterotrophic community was composed of the genus *Bacillus* (dominant species *B. aquimaris, B.*

barbaricus, B. cibi, B. niabensis), whereas the phylum Actinobacteria (genera *Rhodococcus*, *Kocuria*, *Microbacterium*) participated in lower levels. In oligotrophic community a-Proteobacteria prevailed (genera *Methylobacterium, Blastobacter, Bradyrhizobium, Sphingomonas*). The genus *Sphingomonas* has been suggested to be the responsible microorganism to initiate the colonization and formation of biofilms, followed by other Proteobacteria that attach the incipient biofilm. In addition, genera such as *Brevibacillus*, *Ralstonia*, *Bulkhoderia, Acinetobacter* and *Paracoccus* were present. The use of the DGGE method revealed similar fingerprint patterns throughout the samplings. The most noticeable result was the detection of ribotypes in samples derived from the filtering of membranes 0.2 µm, which suggested the occurrence of filterable bacteria.

Concerning the rest of the raw materials examined, *Penicillium chrysogenum* and *Neosartorya hiratsukae* were identified in 3 colorants.

Sunscreen's preservatives were effective against *E. coli* and *P. aeruginosa* but demonstrated moderate effect against *B. subtilis*, which survives and remains detectable after 28 days within the sunscreen. This result in combination with the single strain (*B. safensis*) which was isolated from an unopened bottle of sunscreen and the wide dispersion in the water of genus *Bacillus*, constitutes a factor that needs to be studied more extensively.

P224
Characterization of *Chromobacterium violaceum* alkaline phosphatase coding genes, *phoA*$_1$ e *phoA*$_2$

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Chromobacterium violaceum is a free living, mobile bacterium that lives in usually nutrient-poor environments, such as water sources and soils ofin tropical and subtropical regions. This bacteriuma possesses two annotated *in tandem* genes noted for alkaline phosphatase, *phoA*$_1$ and *phoA*$_2$, whose products share akeep 35% identity withto each other. It is unknown if both gene products are functional or if theyose enzymes have the same biochemical properties. This work has tested conditions needed for induction and detection of total alkaline phosphatase activity in *C. violaceum* and both genes mutations effects. Knockouts of each gene Mutants were constructedbuilt by insertion ofusing a suicidal plasmid carrying an internal fragment of the target gene for homologous recombination and gene disruption. Alkaline phosphatase (AP) activity in the wild-type strain and in the *phoA* mutants was assayed. APTotal alkaline phosphatase activity in the wild-type strain was not induced when the bacteria were grown in minimal medium containing phosphate concentrations higher than 0,1mM phosphate or less. Induction of AP occurred before and independently of growth arrest due to phosphate exhaustionand starvation for this nutrient is not able to induce stationary phase in this bacteria. Both *phoA* mutants presented around 85% less reduced enzymatic activity than the wild-type, but a similar growth pattern.activity in about 85% although the mutations did not interfere in bacterial growth in phosphate limited medium. The maximal recorded AP activity was at total alkaline phosphatase activity has its peak at pH 11, which is quite unusual. The Analysis of proteins PhoA1 and PhoA2 sequences revealed that the residues sites important for enzyme activity are conserved in both proteins. These results suggest that there is evidence that *C. violaceum* has two functional genes for alkaline phosphatase. It was also concluded that *C. violaceum* requires low amounts of phosphate to grow, consistent with the poor environment in which it lives.

P225
Isolation and characterization of a heterologously expressed bacterial laccase from *Geobacter metallireducens*, a strict anaerobe

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Introduction: Bioinformatics has revealed the presence of putative laccase genes in diverse bacteria, including extremophiles, autotrophes and, interestingly, anaerobes. The genome of *Geobacter metallireducens* GS-15, a strict anaerobe, contains five genes for laccase-like multicopper oxidases. Laccases are versatile enzymes that oxidize a variety of compounds using molecular oxygen as the electron acceptor. For this reason, integrity of laccase genes in anaerobes has been questioned.

Objectives: Our objective was to show whether or not one of the predicted genes in *G. metallireducens* GS-15 genome indeed encodes a functional laccase, and if so, to characterize its enzymatic properties.

Materials & methods: Gmet_2154 was heterologously expressed in *Escherichia coli* BL21. The recombinant 6xHis-tagged enzyme was purified by metal affinity chromatography and characterized by spectrophotometric laccase activity measurements.

Results: The purified enzyme oxidized some of the typical laccase substrates, including ABTS, syringaldazine and 2,6-dimethoxyphenol (2,6-DMP). Temperature optimum was estimated to be 60 °C and pH optima for ABTS and 2,6-DMP oxidation were determined to be 5.0 and 8.0, respectively. As commonly observed for other laccases, the enzyme was inhibited by halide anions.

Conclusion: Since the coding region of gmet_2154 seems to be intact, it is possible that this laccase is expressed and physiologically important. However, its physiological role in *G. metallireducens* remains unknown and awaits further studies. This work emphasizes the importance of bioinformatics in finding novel bacterial laccases and confirms the presence of laccase encoding genes in anaerobic bacteria.

P226
Licensing strategies – the basic concern is to achieve the best quality of patent application

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Connecting research and development (R&D) activities with the industry became increasingly important in the last few decades especially at the field of LifeSciences. In order to revert the negative trends of economic growth in most EU countries including Slovenia, the knowledge and intellectual property of public-funded R&D organizations should be implemented into European industry to raise

its competitiveness against other global economies. Technology Transfer (TT) is mainly realized through licensing of patents and know-how to existing and newly created spin-off/spin-out/start up companies. Through the license public R&D organizations receive constant cash inflow from industry, while the companies are allowed to use innovative technologies on the market in return.

The objective of this study was (i) to compare the implementation of Slovenian and European academic research into industry, (ii) to identify the most important obstacles preventing successful licensing of technologies and (iii) to define how to improve licensing trends.

The data on scientific publications, patents, International Search Reports on patentability (ISR) and licensing were obtained by searching the public-available reports on the knowledge transfer, patent databases and internal sources.

The number of patents from Slovenian Research Organizations (SROs) active mostly at the fields of LifeSciences, Materials and Nanotechnologies reached 60-90% of EU average, while the revenues from royalties obtained by licenses reached only 3% of the EU average. This was mainly due to poorly prepared patent applications resulting in the negative ISR within 80-95% of international patent applications filed by SROs. The majority of patent attorneys (PAs) chosen by SROs had poor references with lacking expertise at the technical field of inventions comparing to various PAs from EU and US.

Since weak patents may discourage potential partners from collaboration with public R&D organizations, it is important to choose PAs carefully and with assistance of TT professionals in order to produce patents ready to be successfully licensed in the future.

P227

Caracterisation of *Escherichia Coli* Enterohemorragic O157:H7 in frozen bovine meat in Algeria

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The enterohemorrhagic *Escherichia coli* (EHEC) are responsible for food poisoning leading to hemorrhagic colitis may be complicated by hemolytic uremic syndrome. However, there are currently no government regulations stipulating the procedures for sampling and retrieval of STEC in food.

In this study, we were interested to find the *E. coli* O157: H7 Shiga toxin-producing (STEC) in imported frozen meat. After enrichment and use of selective agents that aim to curb the growth of flora appendix, we have isolated five strains from meat from different countries.

These strains presented the main characteristics of *E. coli* O157: H7, namely non sorbitol fermentation and β-Dglucuronidase negative.

Genetic characterization revealed the presence of toxic genes stx1 and stx2 responsible for pathogenicity of these bacteria through the production of toxins and genes eae and ehxA specific for *E. coli* O157: H7.

The study of resistance of isolates to antibiotics revealed that they are sensitive to the antibioticstested.

Finally the interaction with lactic acid bacteria, shown to all strains were inhibited by the production of bacteriocins.

P228

LuxS-dependent quorum-sensing affects mutation rate plasticity in *Escherichia coli*

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Mutation is the most important of all genetic processes, producing genetic variation on which evolution acts. Previous experiments demonstrated that mutation rates vary between genotypes and locally within genomes. Theory on the other hand predicts that, if the fitness of an organism is high, then it is optimal to minimise mutation rate, but if the fitness is low, then the mutation rate for that same genotype should be increased. How plastic mutation rates can be in nature is however still not clear. We find that rate of mutations conferring rifampicin resistance increases with decreasing absolute fitness of *E. coli* cells. We show that this inverse relationship is mediated by the population density and the underlying mechanism to be *luxS*-dependent quorum-sensing. Given that *luxS*-mediated cell-cell communication is widely conserved in nature, our results might help us better understand the emergence of *de novo* genetic resistance within microbial communities.

P229

Metagenome and metatranscriptome analyses of root-associated bacterial communities

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Interactions between plant roots and their microbiomes determine plant growth and health. In order to study rhizosphere community structure and function, we conducted a metagenomic and metatranscriptomic examination of the rhizosphere effect and plant species-specificity in root bacterial communities. In total, 87.6 Gb of genomic DNA and 47.4 Gb of transcribed RNA data were analyzed from soil and root samples of wheat and cucumber. A non-redundant open reading frames (ORF) database was assembled from the genomic DNA data, containing ca. 2.4 million entries and representing on average 32% of the total genomic DNA reads. Nearly half of the ORFs within the database were identified as bacterial protein coding sequences. Based on bacterial protein coding sequences, the root effect on composition of the bacterial communities was manifested by extensive enrichment of Proteobacteria in both root communities, compared to dominance of Actinobacteria in respective soils. Furthermore, the two plant species selected for distinct taxonomic groups- *Pseudomonas* by wheat and *Cellvibrio* by cucumber. These results concur parallel ribosomal RNA-based analysis of these communities. High-stringency mapping of metatranscriptome sequences to published genomes of populations *Cellvibrio* (*C.*

japonicus) and *Pseudomonas* (*P. stutzeri*) was performed. For both populations, a distinct functional profile was revealed at different niches examined: cucumber roots, wheat roots and respective soils. This was highly indicated by the pattern of RNA polymerase sigma factors. For example, expression level of the sigma 32 was higher for both taxa when colonizing cucumber compared to wheat roots. In *Pseudomonas*, rpoE (sigma 24) was the most highly expressed sigma factor in wheat root, and was expressed at a normalized gene expression levels above six times higher than in cucumber roots. Expression levels of several functional groups of genes and traits, previously identified as critical in plant-bacteria interactions, were selectively expressed by these bacteria on the roots. These include genes involved with denitrification, motility and transport. In conclusion, plant species-specificity was manifested by both distinct patterns of species association as well as by differential transcriptional responses to conditions dictated by the plant genotype.

P230
Mixed target genetic screening of a fosmid metagenomic soil library

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A mix of oligonucleotide probes was used to hybridize soil metagenomic DNA clones spotted on high density membranes. The pooled radio-labeled probes were designed to target genes encoding for enzymes such as chitinases, dehalogenases, bacterial laccases and mobile genetic elements including integrons and insertion sequences. Positive hybridizing spots were affiliated to the corresponding 88 clones in the former library, for pyrosequencing the fosmid inserts. After assembly and annotation, new coding DNA sequences related to genes of interest were identified with a good coverage but a low similarity against closest hits in databases. Aside of the traditional functional-based screening, this work highlights the sensitivity of DNA/DNA hybridization techniques as an effective and complementary way to recover novel genes from metagenomic libraries.

P231
ODoSE – a webserver for genome-wide calculation of adaptive divergence in prokaryotes

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The immense genomic diversity of bacteria and archaea is rapidly being uncovered by next-generation sequencing methods. Much attention in comparative genomics studies is given to differences in gene content mediated by lateral gene transfer, gene duplication and gene loss, as related strains can differ markedly in gene content. However, it has become increasingly clear that bacterial core genes that are conserved between species play a major role in niche adaptation as well.

The McDonald Kreitman (MK) test is a widely used test of selection that compares patterns of non-synonymous and synonymous substitutions within a species to those separating this species from an outgroup species. Adaptive (non-synonymous) mutations can be assumed to contribute more to between-species divergence than to within-species polymorphism because they fix rapidly compared to neutral (synonymous) mutations.

In order to provide a user-friendly method to apply the MK test of selection to entire bacterial core genomes, we have developed a web service with a Galaxy graphical user interface called ODoSE (Ortholog Direction of Selection Engine). The ODoSE pipeline, available atwww.odose.nl, allows researchers to select prokaryote genomes of interest from the NCBI database and/or upload their own genome data, allowing for the genome-wide characterization of adaptive divergence.

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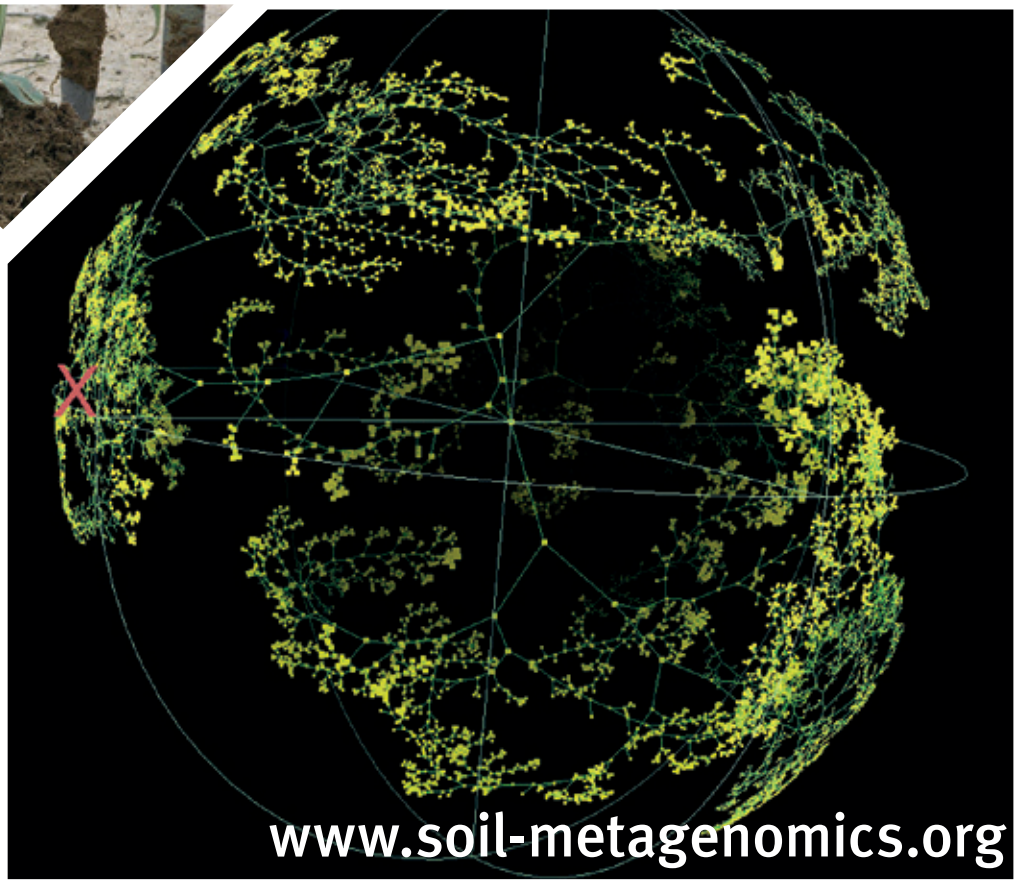

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